

Helping the Feet to Touch the Ground

THE fact that Mr John Davis's subcommittee on science, research and development in the House of Representatives has turned sour on the proposal by the National Science Foundation to mount a programme under the heady title Research Applied to National Needs (see page 8) is a mark of sanity that should help to keep the committee's reputation bright. With luck, this decision may also make the National Science Foundation pause in what seems to have been its recent determination to recruit a larger budget by any expedient. In retrospect, it is a great misfortune that the foundation should have abandoned with such apparent ease its traditional commitment to the support of graduate education and that it should have embraced so lightheartedly the view that it is possible for an organization with its traditions in academic life to set up as a manager of basic research on a large scale. The trouble, of course, is that the foundation has done too much to conform with what must have seemed the prevailing view in Washington—the view that the only scientific research worth having is scientific research that brings social benefits comparatively quickly. To cast doubt on this principle is in no sense to deny that social problems should be attended to or that scientists as such should be especially concerned. There are, however, limits to the speed with which social problems can be solved and there is hardly ever an assurance that research by itself will do the trick. If the National Science Foundation had got its money and had in the process implicitly promised solutions for some of the taxing problems of earthquake engineering or weather forecasting, there is a danger that it might have oversold the prospectus of science in a manner that could only have brought discredit on the foundation and even the profession of science as a whole. Mr Davis's subcommittee has done a public service not merely for American taxpayers but for professional scientists in saying that the National Science Foundation should be less trendy and more devoted to the purposes for which it was set up.

How has it come about that the foundation so badly misjudged its approach to Congress this year? The first thing to be said is that there have been signs of waywardness for at least a year. At the spring meeting of the American Physical Society in Washington in 1970, for example, Dr William McElroy, the chairman of the foundation, uttered in public his belief that if scientists wished to secure a continuing claim on public attention, they should ensure that their proposals for research and development could be seen to be socially relevant, especially in Congress. These were the days in which the foundation set up its luckless programme under the banner Interdisciplinary Research on Problems of our Society (IRPOS). The experience of the past year should have warned the foundation off the still more ambitious scheme called RANN. To be sure, the programme of Research Applied to National Needs includes some work

already under way as well as a number of aspirations still to be made realities, but it is the trick of putting the cart before the horse, of supposing that specifying a problem is a necessary and sufficient condition for its solution, that has caught the foundation at such a disadvantage. Quite apart from the embarrassment of finding that its traditional champions in Congress should have been the first to criticize its budget, the foundation will now find itself having to inflict on its newly created management structure a series of changes which are likely to be unpalatable medicine.

The cavalier way in which the foundation seems to be about to wash its hands of a continuing concern with graduate education is another source of discontent. To be sure, the amount of money available for research in the current year is enormously increased compared with the allocation for 1970, but there is still no sign that the foundation will accept the principle of its own governing body's declaration two years ago that graduate education should be considered a national responsibility. Instead, after huffing and puffing on this issue a year ago, the foundation has in the past few weeks complacently accepted the Administration's view that the difficulty some graduates experience in finding jobs is a sign that there may now be a surplus of highly skilled labour in science and technology. At the same time, it seems to have accepted that there is no longer a need to encourage smaller and less successful universities to develop graduate schools or to increase their programmes of research. Enough, it is assumed, is enough. At the public inquiry this year into the foundation's budget, Dr Philip Handler was entirely right to protest that the foundation had turned its back on one of its chief responsibilities. To be sure, the budget is bigger than ever. It is also possible that in the long run there might be more money for research in a billion dollar budget made rapidly to grow by devotion to the cause of research applied to national needs. Yet the abandonment of the principles embodied in the 1969 report of the National Science Board does more than rob the foundation of its credibility—it is a bad example to the rest of the federal government.

It is hard to avoid the suspicion that the fumbling for policy which has characterized the foundation in the past few months is largely a consequence of its attempts to create a much more powerful central direction for itself. After all, it is now less than a year since the White House vouchsafed the appointment of the statutory complement of senior managers—a deputy director and three assistant directors. The way things have turned out, there are serious doubts whether this arrangement is as much of a benefit as its creators intended. Then, two years ago, the previous chairman of the subcommittee on science, research and development, Mr Emilio Q. Daddario, was plainly anxious to provide the foundation with a handful of senior appointments sufficiently attractive and



sufficiently imbued with the spirit of the in-and-outers who flock to and from Washington. In the event, however, the foundation may have been lumbered with a number of vested interests which will sectionalize its

proceedings and make the allocation of its funds less the consequence of a coherent forward strategy than a kind of compromise between conflicting interests within the foundation's management.

Keeping a Straight Bat

MRS MARGARET THATCHER, Minister for Education and Science in the British Government, succeeded in her appearance before the Select Committee on Science and Technology last week in saying hardly anything that mattered about the future organization of civil research in Britain. As on many previous occasions with many previous ministers, her position seemed to be that the time is not now for public speaking because there is a committee sitting on the problem and due to report some time next month. The trouble, of course, is that there is always a committee sitting and about to report. This time, it is Sir Frederick Dainton and the Council for Scientific Policy which serves as an excuse for saying very little. Mrs Thatcher's trouble, unfortunately, is that there is very little that Sir Frederick Dainton can do to remove the essentially political content of the decisions that she will ultimately have to make. To be sure, an honest man and a hardworking committee can do a lot to throw light on important problems but in the last resort it will be for the government to say how much it is prepared to spend on scientific research, how it wishes to see the research organized and what it expects to get from the expenditure. Mrs Thatcher could, if she had chosen, have given this week a clearer picture of how in the end she is likely to decide.

In the field of competence of the Council for Scientific Policy, the management of funds for the research councils, it is impossible to separate research and higher education. One of the most striking developments in the past few months has been the way in which British university teachers seem passively to have accepted the view that in the years ahead, when funds available for research will grow less quickly than the numbers of students at universities, it will be progressively harder for academic scientists to regard research as the central part of their work. Increasingly, larger proportions of them will have to become dedicated teachers and will have to earn their reputations in that way. The result will be a change in the character of British academic life which, over the years, could modify not merely the nature of scientific scholarship but also the attractiveness of working in universities. Or is it possible, as some academics suggest, that the prospect of less pressure on research may be a relief to some of those now working in universities?

Whatever the truth, the forward estimates of expenditure by the research councils as a whole suggest a growth rate which will diminish from 6.5 per cent a year at present to 3.3 per cent a year (in real terms) three years from now. In circumstances like these, it is of course wise and sensible that the Science Research Council, the chief supporter of scientific research, should resolutely pursue its policies of selectivity and concentration. If resources are no longer sufficient to spread uniformly through the university system, it is obviously better that they should be concentrated at a few places where effective work can be carried out. Mrs Thatcher's problem, hardly likely to be simplified at all by anything that Sir

Frederick Dainton may suggest, will be to know what provision is to be made not merely for the university departments that will find themselves less well provided with research funds but also for the other parts of the higher education system, especially the polytechnics.

A few practical courses of action suggest themselves. Thus it may be possible enormously to increase the amount of research carried on within the academic sector by lumping together those activities of the research councils—the MRC and the ARC as well as the Science Research Council—which are concerned with basic research. Specifically, it should be possible to win great benefits by incorporating many of the basic research units and stations maintained by the Medical and Agricultural Research Councils within universities or polytechnics. The Natural Environment Research Council is an even greater potential source of further power for university research. There is very little doubt that Britain has suffered considerably in the past few years from the way in which research establishments like these have been able to exist in the never-never-land that lies between education on the one hand and industry on the other. The time has now come for change.

It is also time that the British government sought some way of encouraging within the polytechnics work of a kind that would allow these young institutions to build up reputations for themselves as independent centres of learning and scholarship. Too easily, as in some of Mrs Thatcher's own statements in the past few weeks, exhortations to the polytechnics to develop separately from the universities sound very much like exhortations to be satisfied with shorter commons. And finally, the government could do a great deal in its handling of academic matters to encourage flexibility in the careers of teachers in higher education, making it possible for those who choose to teach to win reputations and money in the process.

But would not the merging of the research councils create too monolithic a central sponsor? Are there not advantages at present in the way in which academics are able to play off one research council against another? Unhappily, there is no evidence to support this fond belief. Especially when funds are hard to come by, the research councils work closely in concert with each other. It is true that the special connexions of the Medical and Agricultural Research Councils with the profession of medicine and the agricultural industry respectively give their work a special flavour but this is not a clinching argument for their continued independence. The case for welding them together into a more efficient (because larger) organization for supporting academic research is, first, that there is a prospect that the funds available for such work will fall short of the real need in the years ahead and that a properly organized and more powerful research council would be able to make more rational decisions about priorities in research. As things are, there is no way of weighing in the same balance the potential

rewards of, say, radio astronomy and medical research, nor is there any way of making sure that the funds available are shared fairly among the different kinds of institutions which are clamouring for them. Mrs Thatcher mentioned earlier this week the now familiar argument that the Agricultural Research Council should become a branch of the Ministry of Agriculture and there is a strong case for such a breach of the old Haldane principle

that there should be creative tension between government departments and research organizations. The point that will eventually be borne in on Mrs Thatcher is that the time has probably arrived in Britain when resources are insufficient to support all the research that the separate organizations would like to carry out and at the same time to provide the government departments with good advice.

Does Britain Belong to Europe?

By this time next week, it should be clear what response the European Economic Community will have made to the crucial questions which have arisen about the proposal that Britain and other members of the free trade area should belong to the European community. As it turns out, the most stark issues have been left until the very end, so that it is plain that the past year of supposed negotiations has been hardly better than shadow boxing. Indeed, the discussions in Brussels have been dominated not by the important political questions now to be settled in a few weeks of how Britain and its associates could fit into the institutional framework which has been developed in the EEC in the past decade, nor even by the important economic issues still outstanding such as the industrial objectives to which an enlarged Europe would aim, but by an unseemly haggle about the contribution Britain would be expected to pay towards the unified budget of the EEC. In short, the negotiators have hardly begun to talk about long-term questions but, rather, have concentrated on strictly transitional matters.

What are the implications of membership of the EEC for the scientific and technical communities in Europe, on the mainland but also in Scandinavia and Britain? In the days of Mr Harold Wilson, there was always a great deal of talk of how it would be possible to multiply the effectiveness of industrial research and development within the EEC. And to Mr Wilson and previous Prime Ministers must be given credit for a host of collaborative technical enterprises, some successful and some less successful, which at present litter Europe. The truth, alas, is that technical enterprises will be industrially and commercially more effective in an integrated Europe only to the extent that they have free access to the common market and free access to the sources of capital in Europe. The new system will be more effective in its use of technical manpower only to the extent that in an enlarged community it will be possible for the efforts of a single team in research and development to carry out the work of several competing teams. Inevitably, an integrated EEC would be a place in which the rewards of technical development would be most apparent in the way in which scarce technical resources could be spared for work in fields which Europe at present cannot afford. Those wishing to make fortunes out of the enlargement of the EEC, assuming that it comes off, had better put their money on diversity rather than on the attempts which are conventionally made to bring about international collaboration on familiar projects. That is one elementary lesson to be learned.

The second and more important inference is that membership of the EEC by Britain and its associated countries would in the long run create opportunities for scientific developments which are at present undreamed

of in the patchwork of modern Europe. Although the benefits in technology of a larger community would be the chance to do tasks which are at present off the beaten track, in the long run the benefit would be the way in which the size of the European scientific community was multiplied by, say, three. The benefits here are not merely that a larger pool of technical people is more able to accomplish known objectives but that there are ways in which a larger group of mutually interested people is able to devise schemes which are beyond the reach of others. In this spirit, European scientists must by now be painfully aware that much of the creativity of science in the United States is a function of the way in which kindred spirits from different and distant institutions are able to seek each other out and work together. In the long run, if there is to be an integrated Europe, this is the way in which the European scientific community should be organized. Is it too soon to think of heading in that direction? Would it not be sensible—but perhaps it is now too late—to consider whether freedom of movement within the European university system, the common recognition of professional qualifications, and even access to such funds for research and development as may exist should be regarded as essential elements of a European policy?

100 Years Ago



NOTES

It is stated that the Astronomer Royal is to have the honour of a K.C.B. conferred upon him in recognition of his services in respect to the International Exhibition. We trust this rumour is not strictly correct; for unless it is to be generally understood that services are to be rewarded in the inverse ratio of their value, it is simply grotesque and unbecoming of the Government to ignore all the Astronomer Royal's services to Science, and all his unpaid services to the State in connection with subjects more important to the nation than all the exhibitions which ever have been or ever will be.

It is with very great pleasure that we print the following intelligence of the safety of Dr. Livingstone:—Despatches were received last week at the Foreign Office from Dr. Kirk, the Acting British Consul at Zanzibar, containing information of the safety of Dr. Livingstone in October last. The doctor was then at Manakoso, helpless, without means, and with few followers. Dr. Kirk had sent him supplies to meet his immediate necessities, which, it was hoped, would shortly reach him.

From Nature, 4, 31, May 11, 1871.

OLD WORLD

SELECT COMMITTEE

Worries about Research

THE Select Committee on Science and Technology heard evidence on the research councils this week from the Secretary of State for Education and Science, Mrs Margaret Thatcher. The committee was clearly anxious to find out to what extent the research council system was to be modified but it was largely unsuccessful because of Mrs Thatcher's unwillingness to make any policy statement in advance of the report of the committee of the Council for Scientific Policy under Sir Frederick Dainton on the future of the research councils, which is expected in June.

Mrs Thatcher emphasized the role of the research councils—to "retain an adequate research capacity in all scientific disciplines" and to support fundamental research in such a way that specific research projects and scientists of great promise could be encouraged. It would always be the responsibility of her department, she said, to see that this research capacity was maintained.

Members of the committee were, however, anxious about the reasons for Sir Frederick Dainton's review of the research councils after only about six years of operation but Mrs Thatcher said that the review had been principally occasioned by a strong suggestion from the Ministry of Agriculture, Fisheries and Food that it should take over the responsibilities of the Agricultural Research Council. Such a change could not be made without having a fundamental effect on the other research councils and possibly encouraging other government departments to try to obtain similar control of civil research. If this happened the objective of maintaining an adequate national research capacity would be severely jeopardized and it is therefore vital to look at and update if necessary the research councils and their fields of responsibility.

Sir Harry Legge-Bourke probably expressed the uncertainties felt by the committee and the councils in the clearest terms. Was the Secretary of State fully aware of the way the research councils felt? Mrs Thatcher suggested that there was always concern when an inquiry of this sort was going on but that the research council system should be seen responding to present day requirements—for example, the need for the proper organization of pollution research which cuts across the boundaries of most of the research councils.

On finance, Mrs Thatcher assured the committee that the research councils (which spend about £100 million per

year between them) could continue to rely on the government for support provided that no recommendations are made which would alter the whole structure of the research councils. But if for any reason there were further large cuts in the money devoted to civil research, she said, then the research councils would have to be scrutinized carefully to find out to what extent their work is relevant to "retaining a fundamental research capacity". The Secretary of State agreed with some members of the committee that one solution was to hive off the development work carried on by, for example, the Agricultural Research Council, to the appropriate government department.

During a brief discussion of the work of the Science Research Council, the committee asked Mrs Thatcher whether she thought there was a danger of over concentration if the council continued its policy of selectivity and concentration in the choice of projects. The Secretary of State pointed out that the mounting expense of equipment for scientific research made it absolutely essential that a policy of this type should be adopted but that it was always difficult to make sure that an appropriate balance was maintained.

The committee also brought up the idea that a single minister should be responsible for at least the collection of data on the whole government research effort. In this way, it felt, it would be easier to assess the effectiveness of the whole national research and development endeavour—of which the research councils represent only about 10 per cent—and make coherent plans for the future.

CAFD

Tiger with Paper Teeth

THE Council for Academic Freedom and Democracy (CAFD) made public earlier this week the findings of its most recent commission of inquiry (*Craigie College of Education*, available from the National Council for Civil Liberties, 15p). The report of the commission, which deals with the case of a lecturer whose contract was not renewed, has harsh things to say about the principal of the college and the Board of Governors.

The report of the Craigie affair calls for the Scottish Department of Education to set up an independent Court of Inquiry to examine the whole sorry business—on the face of things a very reasonable proposition but one which seems unlikely to be implemented. A spokesman for the SDE said last week that the department had received a copy of the report, but as the responsibility for hiring and firing lecturers lies

with the college governors, the initiative for such would have to come from that quarter.

Whatever the rights and wrongs of this particular case, the Craigie affair calls into question the activities of such groups as CAFD. Although nobody can seriously doubt that the group, under the able chairmanship of Professor John Griffith of the London School of Economics, is acting from the very highest of motives, any such organization must risk being accused of arrogating to itself the role of judge and jury. This is certainly true of Craigie where, unless some sort of tribunal is set up, the principal and governors must appear to have been convicted without trial and without recourse to further judgment. Indeed, the administration at Craigie is critical of the CAFD report, but it is not yet clear if it will get the chance to ventilate its complaints.

What, then, can be said to justify the CAFD's existence? In the first place, mounting an inquiry and publishing the results are weapons used only as last resorts where all other means have failed. Since the council was established in October 1970, more than 40 applications for help have been considered, but in only four cases have commissions of inquiry been mounted. Three reports have been published, and one, the Atkinson case, is still under consideration. In all the other cases, some kind of equitable settlement has been arranged either by direct negotiations or by interceding with the unions concerned.

What effects have the published inquiries had? The case of Anthony Arblaster is particularly contentious, for the CAFD report stimulated the Association of University Teachers (AUT) to promote its own inquiry, the results of which suggested that all was well and that there was no cause for anxiety. The local CAFD group has now produced another document listing alleged errors and omissions in the AUT inquiry. In spite of this controversy, Manchester set up a Senate committee which has now proposed an entirely new structure for the appointments committee in which professorial staff no longer predominate. Arblaster, denied a post at Manchester on allegedly political grounds, is now employed at Sheffield.

The *Bolton Sackings* (see *Nature*, 230, 420; 1971), the second published inquiry, has had equally fundamental consequences, although the results have been less overt. The commission decided that Bolton Institute of Technology was attempting to provide higher education on the cheap to the detriment of existing and projected courses. It seems likely that some of the embryo degree-level courses may now be

scrapped as a result of CAFD's attentions.

CAFD is also examining the problem of what kind of information should be given in references. Should they contain only matter essential for an assessment of the research and teaching ability of the candidate, or are personal and political details relevant? And there are other matters, such as the need for confidential files, on which the council can comment legitimately and with weight.

CAFD's chief argument is that there is too little democracy in education, especially in the technical and college sectors, and that there is an indisputable need for an independent body to act as watchdog. It seems, for example, that junior members of staff in technical colleges and the like are unaware of their basic rights in matters of teaching and policy, and that there is a great need to publicize the principles of academic freedom. At present the aims and actions of the council seem wholly admirable. But it would be good to see a greater measure of liaison between the council and, say, the DES (which claims never to have received the council's publications), for there is always a need to watch the watchdogs.

GENERATORS

More 500 MW Setbacks

BREAKDOWNS of 500 MW generating sets still seem to be plaguing the Central Electricity Generating Board, in spite of some indications towards the end of last year that many of the problems had been solved (see *Nature*, **228**, 1246; 1970).

The breakdowns during the winter of 1969-70 were the subject of an inquiry by the Select Committee on Science and Technology (HMSO; 20p) during which it was revealed that as many as twelve of the 500 MW sets were out of use at times near the end of December 1969, when the board was obliged to make 6 per cent voltage reductions on a nationwide scale and the total lost output was 3,670 MW.

More recent figures, for March 15, 1971, show that of thirty-five sets at some stage of commissioning or synchronization, eleven were not available for the delivery of power to the grid. In a few of these cases, the reason is probably a routine inspection, but even a conservative estimate suggests that between 20 and 30 per cent of the 10,000 to 15,000 MW which could be expected from all the sets (taking into account their differing stages of commissioning) was lost on that day, and there is no reason to think that this is atypical. This level does not seem very different from that reported at the end of 1969 and suggests that, far from

being solved, the problems may have become worse.

One crumb of comfort, however, is that the fully commissioned generators at West Burton, which figured among those out of use during the voltage reductions in December 1969, do not appear on the list of those out of use on March 15, 1971. On the other hand, commissioned generators at Eggborough, Ferrybridge (C), Cottam and Ironbridge (B) were out of action in both December 1969 and at March 15, 1971. Of the generators synchronized, but not commissioned at the end of December 1969, two at Aberthaw B still seem to be giving trouble.

The CEBG plans to introduce eleven more 500 MW sets by the end of 1972, and the larger 660 MW sets are to be incorporated into the Hinckley Point B, Hartlepool and Dungeness nuclear power stations. The fate of these larger sets will depend on the extent to which the CEBG has taken to heart the recommendations of the Select Committee which suggested, for example, that design contracts should always be placed for steam raising plant, and that plant should be tested whenever possible before attachment to the national grid.

The troubles with the 500 MW sets have also contributed to the £30 million bill for repair and maintenance, which the CEBG has had to foot during the past year, and were obviously an important factor in the estimated loss of more than £10 million which the board is thought to have made. It can only be hoped that continuing troubles with the 500 MW sets will not become, as seems possible, a permanent feature of the British electricity supply industry.

NUCLEAR POWER

Ship Comes Home?

THE British government's confirmed belief that nuclear shipping is uneconomic was repeated last week by a committee set up by the Department of Trade and Industry six months ago (*Report on the Nuclear Ship Study*, HMSO, 70p). The report itself is the work of a study group set up by the old Ministry of Technology after an approach from shipbuilders anxious to find out whether nuclear shipping would have a future. The nub of its essentially sceptical commentary on the question, consisting largely of a detailed calculation of the comparative performance of nuclear shipping and oil-fired vessels, is that nuclear reactors are by comparison with oil-fired turbines uneconomic and economically risky.

The report of the committee says that pressurized water reactors with low enrichment fuel are the best sources of power. Many readers of the report will

be surprised to learn that some of the most advanced studies so far available have sprung from the Anglo-Belgian Vulcain project, which has in particular suggested that the time to develop a 40,000 horsepower unit for driving ships will be comparatively short. Even so, it is reckoned to be 7½ years from the start of a development programme to the commissioning of a prototype ship, and production of commercial ships would follow two years later. The cost of a prototype development and construction programme is reckoned to be about £3 million spread over a period of several years—a calculation which assumes that a prototype ship of 40,000 horsepower could be made to earn some revenue as well as to consume nuclear fuel. Similar calculations for conventionally powered ships suggest that they are cheaper to build and operate than nuclear vessels, and in particular freight costs of nuclear shipping would be between 24 and 40 per cent greater than for conventional vessels.

That said, it is unlikely that the latest repetition of the conventional wisdom on nuclear shipping will banish for good and all the optimism of those who argue that in this economic study, it is pointless to compare like with like and that, in particular, the economic advantages of nuclear shipping are the ease with which fast ships can be built and the way in which, in operation, the turn-round time can be cut.

BSSRS

Roses On Their Way

HILARY and Professor Steven Rose this week announced their resignation from the committee of the British Society for Social Responsibility in Science. Mrs Rose, who has been chairman of the society for only a few months, and her husband were very much to the fore in laying the groundwork for the policies and progress of the BSSRS. Many aspects of the development of BSSRS have been coloured by their views and there can be no doubt that the society must change in many ways now they have given up the reins.

There have been whispers that the Roses and the rest of the BSSRS committee have not been seeing eye to eye on matters of policy, but Mrs Rose says that disagreements of this sort have played no part in her sudden resignation in mid-session. Having seen the society through the early and difficult years, the Roses say they now feel justified in off-loading some of their committee work to concentrate on writing and other chores.

NEW WORLD

Academy Squirms a Little in Sudden Spotlight

by our Washington Correspondent

THE annual meeting of the National Academy of Sciences is usually a quiet affair of interest only to the new members who are elected and the hopefuls who have to wait another year. This year's meeting, held last week in Washington, was different. One member resigned, and three threatened to do so shortly, in protest at the academy's acceptance of work on projects classified as secret. The election procedure was marred by an incident in which a scientist who would otherwise have been elected was passed over because of a popular article he had written. A third hazard, more successfully negotiated, was the attempt of one member to manoeuvre the academy into a position that would seem to countenance his racist views.

These stresses and strains reflect in part the academy's growing role, half sought, half thrust upon it, as an adviser and consultant to the government. The president, Philip Handler, and other academy leaders have made valiant efforts to refashion some of the academy's procedures so as to suit it for a more vigorous role, but the new activism has created problems of its own. One of them is the public position taken by Handler against outspoken or alarmist statements by other scientists on issues such as environmental pollution.

Handler's position, which probably commands the sympathy of most members, is that the academy in its adherence to the scientific facts should avoid the use of the diatribe, hysteria or hyperbole in bringing these facts to public attention. This attitude has been interpreted by the academy's critics as an attempt to repress criticism. For example, the former Secretary of the Interior, Stewart L. Udall, whose assault on the academy last December has done much to awaken public interest in its affairs, has criticized Handler for attempting to put down scientists who speak out on environmental issues (see *Nature*, **229**, 151; 1971).

The views of Handler and the academy council on the dangers of environmental hyperbole form the background to the non-election to the academy last week of LaMont C. Cole, professor of ecology at Cornell University. Cole is the author of a hypothesis, first published in the magazine *Bioscience* and more recently in *Environment*, that continued poisoning of the marine phytoplankton by

industrial chemicals will lead to a depletion of the world's atmospheric oxygen which, over the course of about 3,000 years, could result in a total reduction of some 40 per cent. The hypothesis is not without its critics, but in any case it seems to have been Cole's previous work, mostly on insect population dynamics, which led to his being proposed as a candidate for membership to the National Academy.

The academy's election procedure is one that attracts epithets, even from members, such as "baroque", "bizarre", "neither simple nor strikingly democratic". In rough outline, it consists of arranging the top 75 candidates in rank order according to the number of votes received. The rank order is then re-juggled so that no more than 18 physical scientists, 18 biological scientists, and 10 applied scientists shall be among the first 46 names, which are automatically voted in. The remaining four places to make up the annual entry of 50 new members lie in the gift of the academy's council, and are used to reward yeoman administrative services and the like by people whose scientific eminence would not normally qualify them for membership. If there are no such gold watches to be handed out, the council's usual practice is to nominate the candidates ranked 47th to 50th.

In last week's election rites, Cole received a total of some 142 votes which placed him about 44th in the first roster. After rearrangement to satisfy the disciplinary quotas, Cole's name slipped to 47th place, which ordinarily would have assured him of election. However, when the new Home Secretary of the academy, Allen V. Astin, read out the names of the four council nominees, Cole's supporters were surprised to hear the names of the individuals ranked 48th to 51st on the order list but not that of the 47th. According to various accounts of what went on in the closed meeting, an academy member then suggested there had been some mistake. Astin replied there had been no mistake in the council's proposals. Cole had been passed over because of doubts in the council about his scientific competence. Astin was apparently then asked how many members of the council called themselves ecologists (answer: none), and by what right the council presumed to judge unworthy of membership a candidate who had received 142 votes from the members.

At this point the council position was re-explained—it was not Cole's scientific competence that was in doubt, but the fact that he had made alarmist and scientifically doubtful pronouncements about the relationship between the burning of fossil fuels and the depletion of the world's reserves of atmospheric oxygen. One of Cole's supporters rejoined that this argument had been put forward in a popular article which should not be considered part of the scientific work on which his admission should depend. This defence somewhat backfired because of the counter-argument put forward by Handler that scientists should be even more careful about what they say in public than when addressing themselves to scientists. Following this debate the council's nominees were accepted and candidates 1 to 46 and 48 to 51 were by due process elected members of the prestigious National Academy of Sciences.

At a press conference held after the election meeting, Handler said he had neither opposed nor supported Cole—the president has no vote—but that "I said it behoves scientists to be even more sure of their facts when speaking to public bodies than when speaking to scientists who can challenge them". Astin, asked last week about ecological expertise among the council, said that no member calls himself an ecologist, but John W. Tukey, a mathematician, is skilled in atmospheric science, and Clement L. Markert and Philip Handler, both of them biologists, are "knowledgeable in the area of ecology".

A less byzantine piece of business was the academy's response last week to the motion raised by William Shockley, co-inventor of the transistor, that the academy should urge the nation to study the "hereditary aspects of our national human quality problems". Shockley's belief is that blacks are genetically less intelligent than whites, and at his insistence the academy appointed two years ago a committee under the demographer Kingsley Davis of Stanford University to consider the issue. The committee recommended closer interdisciplinary cooperation among researchers with regard to behavioural genetics, consultation by the National Science Foundation with other federal agencies about the "possible educational implications of human behavioural genetics", and the setting up by the academy of another committee to study long range research on

the subject. The membership snubbed Shockley by voting in favour of the first proposal but against the second two.

Of rather greater import than either the exclusion of Cole or the repudiation of Shockley was the resignation from the academy of Richard C. Lewontin, a geneticist at the University of Chicago. The reason for Lewontin's resignation is what he sees as the irreconcilable contradiction between the acceptance by the academy of secret work and the responsibility of members for all work carried out in the academy's name, which means that members are put in the position of being responsible for work they are prevented from even seeing.

In his letter of resignation delivered last week (and not yet formally accepted by the academy), Lewontin stated that the secret research—which amounts to between 2 and 5 per cent of the total work undertaken each year—was allowed “in the interest of a small group of Academy and National Research Council functionaries who have a personal interest in playing an important role in government”. Despite his “repugnance” at continued membership in such a body, Lewontin explained, he had remained a member of the academy hitherto “in the hope of helping others to see the problem. I have failed in this effort”.

Lewontin's motion that the academy cease to advise the government on secret work was tabled at last week's meetings (which means voted down without debate), with only about a dozen members voting in his favour out of some 250 that were present. Only four or five supported Lewontin when he introduced the same motion at last year's meeting.

Lewontin's efforts on this issue are nevertheless probably rather more important than the number of his supporters might suggest. The act of his resignation may well induce other members to face up to what is certainly an incontrovertible, if purist, objection to the academy's procedures. Moreover, Lewontin has the support of several younger members of the academy, three of whom have stated their unwillingness to remain in the academy much longer if the issue raised by Lewontin is not resolved soon. The members are Thomas Eisner and Bruce Wallace of Cornell University and Robert MacArthur of Princeton. At last week's meeting, Handler stressed the importance of persuading younger scientists to sit on the various committees of the National Research Council, the operating arm of the academy but, as one member of the academy said last week, the chances of recruiting young scientists will be diminished if the academy itself loses a large proportion of its younger membership. The average age

of the academy is 62, and a quarter of the members are over 70. Lewontin, by contrast, is 42, and the three colleagues who have threatened to resign in sympathy are aged 41, 41 and 50.

Lewontin's confrontation with the academy began about 2½ years ago when he asked that members should be told more about the activities of the National Research Council. In the course of these inquiries he discovered the little known (though not secret) fact that the academy undertakes classified work. Persistent efforts to get the academy to drop secret work have been rebuffed, though not entirely ignored, by the leadership. Lewontin said last week he is resigning not necessarily because he sees no hope of a change in the academy's position but because “for my stand to be realistic there's only a limited amount of time for which I can continue to remain a member”.

There can be little doubt that Lewontin has put his finger on an uncomfortable paradox in the academy's constitution. Although the academy's charter, signed by President Lincoln, enjoins it to undertake work for the government, classified work, security clearances and the like did not exist in Lincoln's day. Moreover, the academy's yearbook makes explicit that “By authority of its Congressional charter, the membership of the Academy of Sciences is ultimately responsible for all the work done by the overall organization.”

An answer to this paradox, with which most members presumably go along, has been furnished by academy theoretician Harvey Brooks, dean of engineering at Harvard and chairman of the academy's Committee on Science and Public Policy. The answer, Brooks argues, is that since the National Research Council in any case puts out far more reports than any individual can read, the membership in effect delegates its authority to committees appointed for the purpose, in particular the Report Review Committee chaired by George B. Kistiakowsky.

The academy has at least made a just perceptible move in the direction of Lewontin's proposals. Last week Handler announced that more would be done to keep members informed of the reports produced by the National Research Council, and that classified reports would be open to inspection by members having security clearance (between a third and a half of the membership) together with a “reasonable need to know”. At a press conference held after the meeting at which Lewontin's motion was tabled, Handler and Kistiakowsky discussed various aspects of the academy's handling of secret work. Kistiakowsky, who has undeniable credentials as an anti-

militarist—he severed all connexions with the Department of Defense in 1967 because of the escalation of the war in Vietnam—said that for the academy with its tradition of giving advice in wartime to refuse work that contributes to the defence of the country would be an abnegation of citizenship. As it is, the academy refuses all work classified as top secret because even the titles of such projects could not be discussed by the council of the academy, which decides what work to take on. In accepting contracts, whether classified or otherwise, the council applies three criteria—first: is there a high enough technical content to make the academy's expertise necessary? Second: are there other suitable organizations which could do the job as well? Third: does it make sense to undertake the study at all? By the end of the present fiscal year, Kistiakowsky said, the academy would have produced a total of 14 classified reports, most of them for the Department of Defense and one for the Office of Emergency Preparedness.

If the academy leadership this year has chosen once more to duck the issue of classified research, at least a positive step has been taken towards broadening the character of the membership so as to include more medical and social scientists. The step is being taken not so much to acknowledge eminent scientists in these fields—though that is part of the reason—as to improve the academy's ability to advise the government on issues falling within the competence of these disciplines. At last week's meeting the membership approved a proposal to raise the annual intake of new members from 50 to 75 next year, 100 in 1973, then dropping to about 60 a year by 1977, an intake that will maintain a steady state membership of 1,200 (the present membership is 900). In order to build up the number of medical and social scientists in the academy, quotas of 12 and 13 respectively have been allotted for these disciplines in next year's intake.

An immediate decision now facing the council of the academy is how to deal with Lewontin's resignation. Lewontin's action is the first time a member has resigned on a point of principle, at least in recent memory, although the physicist R. P. Feynman resigned two years ago for personal reasons. First reactions on the part of the academy leadership to let Lewontin leave without demur may be tempered by awareness of the newly aroused public interest in the academy's affairs. The consumer advocate Ralph Nader, for example, is sponsoring an investigation of the academy through his Center for the Study of Responsive Law—the study, expected to be ready next January, is being undertaken by Philip M. Boffey, an experienced

journalist formerly on the staff of *Science*. Within the academy there are many members who would regret Lewontin's resignation. "Lewontin is an exceedingly able and brilliant man, and the academy should not let him go," a supporter of Lewontin's position on classified research said last week, and "the officers of the council should set about resolving this matter entirely, and say they are not acting on his letter of resignation." Handler, however, who has been scrupulous in allowing Lewontin's frequent and usually acid criticisms to come to the attention of the membership, may feel that Lewontin has already had a fair run for his money. Nonetheless, the academy's standing among many younger scientists would probably be more enhanced if Lewontin were to be persuaded to remain a member and Shockley expelled.

NSF

RANN Cut Down to Size

by our Washington Correspondent

THE National Science Foundation has been only partly successful in its gambit of swinging nearly \$100 million worth of extra funds through Congress under a newfangled and variegated umbrella called RANN. Last week, the Science and Astronautics committee of the House of Representatives told the foundation to reassign to its educational programmes the money they had been stripped of in order to bolster the RANN scheme. The committee also compartmentalized the foundation's budget so that funds cannot later be juggled around to thwart the committee's intentions. Subject to these reallocations, the committee has authorized the House to vote the full \$622 million budget for the NSF requested by the Executive.

These actions are the recommendations of the committee's subcommittee on Science, Research and Development which since March has been holding hearings on the NSF budget under its new chairman, John W. Davis of Georgia. The \$622 million being requested of Congress for the fiscal year starting in July represents a substantial increase over the \$506 million the foundation received last year. Of this increment, \$74 million is for the support of projects transferred from or abandoned by other agencies, but even so, the foundation came out of the budget-making operation last February rather better than other agencies, a position for which it was required to make certain sacrifices.

Chief of these was the castration of its educational and training pro-

grammes; support for institutions was chopped from \$34.5 million in 1971 to \$12.0 million and the science education support programme was reduced from \$100.6 million to \$77.3 million. The rationale for these economies is the official belief that the supply of scientists is now sufficient and there is no need to expand production. The surplus so created has been funnelled into applied science projects, lumped under the title of Research Applied to National Needs or RANN.

The National Science Foundation is customarily thought of as the patron of basic research, in contrast with the mission-oriented agencies which support applied research. But foundation officials have made increasingly bolder use of a loophole inserted into the foundation's charter in 1968 which sanctions the support of applied research. When justifying a budget, first before the Office of Management and Budget and then before Congress, it is easier to defend applied research than the purer sort, which may have much to do with the abrupt escalation in the NSF's support of applied research, from nothing in 1969 to \$12 million in 1970, \$34 million in 1971 and a proposed \$81 million for the coming fiscal year.

The \$81 million for RANN is nearly a third of the total funds assigned to scientific research project support. (The \$257.8 million assigned to the latter programme, however, includes \$27.6 million for engineering and \$12.8 million for materials research. Counting these with RANN as applied research, the

NSF's proposed budget for 1972 contains \$121 million for the support of applied research projects and \$217 million, or less than twice that, for the sustenance of basic research.) The growth of the RANN programme has this year been made at the expense of the educational programmes, and it is this shift that the House subcommittee has reversed.

The subcommittee, supported by the parent Science and Astronautics committee, has lopped \$30.6 million off the RANN programme and another \$11.7 million off the scientific research project support. These savings have been redistributed to institutional support, raised by \$16.8 million to \$28.6 million, and science education support, bumped up by \$22 million to \$99.3 million. One reason for cutting RANN down to size was the subcommittee's feeling that for what was still an experimental programme RANN was growing too fast for its own good. No one has forgotten the fiasco the NSF got itself into with the last major test of its managerial skills, the Mohole project. The subcommittee was also impressed by the volume of mail received by Congressmen asking for the education grants to be restored.

Reservations about the size of the RANN programme were also implicit in the remarks made before the committee by Philip Handler, president of the National Academy of Sciences and a member of the National Science Board, the governing body of the National Science Foundation. "For my part," Handler told the subcommittee

NSF: Patron or Tyrant?

PUBLIC warning that the NSF's new-found fondness for applied research may lead it to browbeat the academic community into going along with these policies was delivered last week by Frederick Seitz, president of Rockefeller University and former president of the National Academy of Sciences. Speaking to a meeting of the American Chemical Society in San Juan, Puerto Rico, Seitz confessed to having "several grave misgivings with respect to the current enthusiasm for mission-oriented research which is emerging in Washington". Two instances cited by Seitz were the National Science Foundation's growing support of applied research and the present campaign for a crash programme on cancer.

The change in the charter of the NSF allowing it to support applied research, Seitz said, "may well bring about a widespread change in the relationships between the scientific officers in the agencies and the scientific community. The former may well begin to feel a far greater sense of command and be much more inclined to insist that the scientific community do its bidding". Seitz also doubted whether the NSF, whose dealings up to now have been primarily with the academic community, can hope to develop really effective working relationships with industry.

Commenting on the demands for an attack on cancer equal in intensity to the effort to construct the first atomic bomb, Seitz told the Chemical Society he doubted if basic knowledge about cancer justified the expenditure of large sums devoted primarily to applied objectives; it might be more profitable to focus "a substantial part of the support on cell biologists and molecular biologists who remain thoroughly mindful of the cancer problem in their fundamental research".

last month, "I view RANN and its predecessor (the programme was known last year as IRPOS) as a large experiment . . . IRPOS has had no great successes of which it can boast—I hope the reason is only that it is young. Today, one cannot make any great claims that it has really solved a major problem which is pressing on our society."

Handler was also the only witness before the subcommittee to criticize the Administration's throttling of the NSF education programmes, particularly the fellowship and traineeship programmes. "I consider taking money from these two sets of programmes as a trend in the wrong direction. Both are on the road to extinction if this set of recommendations in this year's budget are indeed implemented, and I hope this would not be the case," Handler told the subcommittee. The form of the programmes was laid down two years ago by the National Science Board, of which Handler was then chairman. Evident from Handler's testimony, though not made explicit, was his disinclination to countenance the board's volte-face on this issue.

Of the four congressional committees that have to approve the NSF's budget (an authorization and appropriations committee in both House and Senate), the House Appropriations committee is the one that wields the greatest influence. Authorization committees are often regarded as special pleaders for the agencies with which they deal, and in the case of the National Science Foundation the House Appropriations committee has regularly slashed the annual budgets authorized by the Science, Research and Development subcommittee.

The usual sequence has been for the latter committee to authorize much more for the foundation than the President has requested in his budget and the Appropriations committee to appropriate much less. With new chairmen heading the relevant subcommittees, this year's scenario promises to be less dramatic. Davis's subcommittee apparently toyed with the idea of voting the foundation some \$44 million above the Administration request but decided this would be a face-losing gesture in view of the certain refusal of the appropriations committee to match such an increase.

The relevant subcommittee of the House Appropriations committee, the HUD-Space-Science subcommittee, has in Edward P. Boland a chairman who, if not yet a proved friend of science, is at least prepared to make amicable remarks about the foundation in public (see *Nature*, **230**, 9; 1971). The indications are that Boland's subcommittee will vote the foundation much the same size of budget as the Administration is requesting.

New Academy Members

FIFTY new members were received into the bosom of the National Academy of Sciences at its annual meeting in Washington last week. In principle the members are elected on merit, subject to restrictions on the number of candidates from various disciplines. This year there was a quota of 18 for physical scientists, 18 for biological scientists and 10 for applied scientists (of both the physical and biological variety). The manner of appointment of the remaining 4 members is described in the accompanying article. The 50 new members are as follows:

Edward A. ADELBERG, microbiologist, Yale University; Julius AXELROD, pharmacologist, National Institute of Mental Health; Lawrence BOGORAD, biologist, Harvard University; William F. BRACE, geologist, Massachusetts Institute of Technology; Arthur M. BUECHE, General Electric Company; Allan M. CAMPBELL, biologist, Stanford University; Marvin CHODOROW, electrical engineer, Stanford University; Arthur D. CODE, astronomer, University of Wisconsin; Philip P. COHEN, physiologist, University of Wisconsin; Mildred COHN, biophysicist, University of Pennsylvania.

George B. DANTZIG, operations research and computer science, Stanford University; Don U. DEERE, civil engineer, University of Illinois; Frank J. DIXON, pathologist, Scripps Clinic and Research Foundation; Kenneth O. EMERY, Woods Hole Oceanographic Institution; Josef FRIED, biochemist, University of Chicago; Alan GAREN, biophysicist, Yale University; Riccardo GIACCONI, American Science and Engineering, Inc.; Eleanor J. GIBSON, psychologist, Cornell University; Ward H. GOODENOUGH, University of Pennsylvania; Luigi C. GORINI, bacteriologist, Harvard Medical School.

Harry B. GRAY, chemist, California Institute of Technology; Ernest M. GRUNWALD, chemist, Brandeis University; Arie J. HAAGEN-SMIT, chemist, California Institute of Technology; Norman HACKERMAN, Rice University; Vladimir HAENSEL, Universal Oil Products Company; David S. HEESCHEN, National Radio Astronomy Observatory; David H. HUBEL, physiologist, Harvard Medical School; William P. JENCKS, biochemist, Brandeis University; Michael KASHA, chemist, Florida State University; Robert P. KRAFT, astronomer, University of California, Santa Cruz.

Hans W. LIEPMANN, aeronautical engineer, California Institute of Technology; Irving M. LONDON, physician, Albert Einstein College of Medicine; Peter R. MARLER, animal behaviour, Rockefeller University; Philip MORRISON, physicist, Massachusetts Institute of Technology; Jürgen K. MOSER, mathematician, New York University; Earl L. MUETTERTIES, E. I. du Pont de Nemours & Company; Edward P. NEY, physicist, University of Minnesota; William A. NIERENBERG, physicist, University of California, San Diego; Irvine H. PAGE, Cleveland Clinic Foundation; William D. PHILLIPS, E. I. du Pont de Nemours & Company.

Frederic M. RICHARDS, biophysicist, Yale University; Robert G. SACHS, physicist, Argonne National Laboratory; John R. SCHRIEFFER, physicist, University of Pennsylvania; Richard E. SCHULTES, botanist, Harvard University; Nevin S. SCRIMSHAW, nutritionist, Massachusetts Institute of Technology; Oliver SMITHIES, geneticist, University of Wisconsin; Hewson H. SWIFT, zoologist, University of Chicago; John G. THOMPSON, mathematician, Cambridge University; Sidney UDENFRIEND, molecular biologist, Roche Institute; Gerald J. WASSERBURG, geophysicist, California Institute of Technology. In addition the posthumous election was announced of Otto Laporte, physicist at the University of Michigan at the time of his death.

The National Academy last week also elected the following 10 scientists as foreign associates:

Aage N. BOHR, physicist, Niels Bohr Institute, Denmark; Donald E. BROADBENT, psychologist, Cambridge, England; Augusto GANSER, geologist, Geologisches Institut, Switzerland; Dorothy C. HODGKIN, crystallographer, Oxford, England; Aharon KATZIR-KATCHALSKY, chemist, Weizmann Institute of Science, Rehovot, Israel; Vladimir I. KEILIS-BOROK, geologist, Academy of Sciences, USSR; Frantisek SORM, chemist, Czechoslovakia; Bengt STRÖMGREN, astronomer, University of Copenhagen, Denmark; Armen L. TAKHTADZHIAN, botanist, Komarov Botanical Institute, USSR; Vincent B. WIGGLESWORTH, entomologist, Cambridge University, England.

The new elections bring the membership of the National Academy of Sciences up to 900 and the number of foreign associates up to 117. Allen V. Astin, a former director of the National Bureau of Standards, was elected Home Secretary of the Academy, and the following 4 members were elected to the 12-man council that governs the academy and helps direct the National Research Council: Konrad E. Bloch of Harvard, Robert E. Marshak of the City College of New York, John R. Pierce of Bell Telephone Labs, and Harrison Shull of Indiana University.

UNIVERSITIES

Wiesner at MIT

from our Cambridge Correspondent

In an entertaining article in the *Atlantic* for April, Warren Bennis, the distinguished sociologist, describes the experience of being taken to the finishing post in a contest for the presidency of Northwestern University and then being dropped without so much as a word to tell him he didn't have the job. The story is good, but particularly memorable are his pithy comments on what it takes to be a university president in the United States these days. The turnover is alarming—most presidents of the best schools seem happy to drop out in five years as the wheels of the university grind ever finer. Bennis declares that "presidents are currently harder to find and keep than domestic help". He compares the job unfavourably with that of an ice hockey referee.

And yet American universities still unanimously believe in the post of president. The reputed half million dollars that Harvard spent in finding Professor Derek Bok testifies to that belief in the need for a chief executive. This contrasts with Britain, for example, where many academics are accustomed to vice chancellors whose greatest impact in office may be an injunction to students not to throw fireworks on Guy Fawkes day and whose greatest service is to confer degrees on a few thousand people every year. But it probably reflects a more general difference in style between the British reliance on a powerful civil service and network of high level committees and the American desire to have a helmsman and indeed at times actually to allow him to take the wheel and steer the boat himself.

In the excitement of the Harvard race, MIT's impending change seems to have created barely a ripple. Yet the next few years are going to be crucial for a school with a scientific and technological reputation unrivalled in the United States. The truth is that the decision was predictable from the start and no candidate could have mustered anything like the support that was behind Dr Jerome Wiesner, President Kennedy's scientific adviser, at present provost of MIT.

The retiring president, Dr Howard Johnson, has an enviable reputation. He is highly rated throughout the country and if there were a prize for Most Valuable President he would have it—not for fund raising abilities but for a seeming capability to satisfy most constituencies with well worked out solutions. Johnson is not a man given to bold public gestures and perhaps this has helped him during the past few years when students have rampaged. Most of MIT genuinely felt sorry when

Johnson had to encounter student confrontations—sorry rather than angry or gleeful. One student newspaper wished him charisma as a Christmas present last year. Perhaps he has been better without it.

Wiesner will obviously be a very different president, and the difference will not only be possession of charisma. Most American politicians running for election like to leave their policy statement as "you know where I stand", which saves them a lot of time and thought. Wiesner can really say that with justification. His visibility on campus, his facility at public speaking and the cogent opposition he helped organize to the ABM all speak in his favour in liberal Cambridge. Probably the only really effective arms control measure since the war—the partial test ban—came about during his time in Washington, and the problems and arguments which surround disarmament are clearly an abiding concern of his.

What will Wiesner do for MIT? During a recent interview with him, it was clear that he would play, consciously or unconsciously, a therapeutic role in the technological community. He has a clear idea of the importance of science and technology for society. This strong conviction will obviously be in considerable demand to stiffen the resolve of hard pressed scientists and to find new growth points for the institute, especially at a time when no less a luminary than I. F. Stone can write that technology is being kept in its place by the recent Senate SST rejection. He is fully aware of the times ahead. "Holding the line" was a phrase which occurred several times. Science funding is something which seems to have a periodicity and it is imperative that the institute should remain healthy for the next three or four years beyond which he had hope that a new cycle would be under way.

He voiced concern at the way funding for fashionable subjects was so fickle. A recent example of this is international studies. Money for the Center for International Studies, until a few years ago forthcoming on a large scale, was drying up. Maybe federal support on a broader level are necessary. There is, of course, no University Grants Committee in the United States, and private universities often have a job keeping up with the changing demand and fashion without ditching thoroughly viable and well established subjects.

What about the humanities at MIT? Are they viable? Wiesner spoke of a near crisis in the humanities—an identity crisis which could be compared to the perennial problem of the role of philosophy in the world of learning. In the past year or two, the humanities had come in for much criticism and

resentment for the attacks of some of their number on the institute. Wiesner will clearly have to continue to walk this tightrope for some time to come.

A ground swell of feeling in the institute, much of it fostered by the humanities, had led to the Pounds Panel and President Johnson's decision that the institute should divest itself of the Instrumentation (Draper) Laboratory, while retaining the Lincoln Laboratory attachment. Wiesner was clearly sorry to see Draper Laboratory go, however inevitable and rational it may have been, and he expressed strong hopes that a workable association could remain with Lincoln Laboratory. He was at pains to point out that the divestment decision would have been reached in the normal process of affairs and regretted the appearance that MIT was bowing to the "mindless assaults" that characterized so many campuses in 1969 and 1970.

If any city in the United States is an intellectual hotbed, it is Boston, with its 120,000 students at institutions as diverse as Harvard and Dunkin Donuts University. Could any changes be expected in MIT's relationship with other universities? He was cautious about anything closer than collaboration in individual fields. MIT has very strong links with Wellesley College and many joint programmes with other universities, notably Harvard, in expensive subjects like high energy physics and radio astronomy. But there is no thought of closer relationships, even mergers, to take advantage of the proximity of so many academics in Boston. Wiesner plainly sees competition as an important and healthy factor in the development of university research. He would like to see the various universities continue to vie amongst themselves for academic honours.

The drop in the numbers of students turning to science as a career—a much larger swing in the United States than in Great Britain, for example—did not worry him very much. He saw the quality of students as having changed little—those who were not turning to science were those who in former years had simply used science as a means of getting the degree needed for a profitable career. The students who are now staying in science are those who are really excited by it. Perhaps this is typical of Wiesner's approach to all things scientific—a confidence in the present growth and future strength of science being basically vital to society, and a firm conviction that science is still an exciting thing to do. At a time when scientists and technologists are in some indefinable way losing their status, Dr. Wiesner will have a pivotal role to play in sustaining the momentum of scientific development.

NEWS AND VIEWS

Chemistry in Unfamiliar Places

SINCE the first lunar samples were returned by the flight of Apollo 11, it has been clear that the microchemistry of the lunar surface would turn out to be well worth the trouble of mastering the intricate techniques now being deployed in dozens of laboratories. The report on page 29 of this issue of the analysis of dust from the lunar surface for methane and other carbon compounds is a striking example of how revealing this work can be. What Cadogan *et al.* have done is to show that some of the carbon in their lunar material is in all probability present as methane. In all the circumstances, the amounts of this gas are quite considerable—a few parts per million by weight. As earlier investigations have shown, there is a positive correlation between the amount of methane in the dust and the total carbon content. On this occasion, it also turns out that the amounts of the various carbonaceous materials in the different samples are correlated with the degree of exposure to particle radiation as measured by the proportion of grains with ionization tracks in the outer skin of 100 Å or so. All this ties in neatly with the hypothesis that the samples most heavily laden with carbon are those which have been exposed for longest to the solar wind. The assumption is that carbon atoms are deposited individually at the points at which they are brought to rest in the crystal lattices on which they impinge. Given the likelihood that the solar wind will saturate the exposed dust on the lunar surface with atoms of hydrogen, 10,000 times as abundant as carbon atoms, it is not difficult to see how molecules of methane might arise from the products of the solar wind. The interest of the work now reported is that it has been possible to demonstrate that there is also methane, perhaps in concentrations very much smaller than in the dust, in the indigenous rocks on the surface of the Moon. Is this, as Cadogan *et al.* suggest, the remnant of the primordial gas from which the Moon was formed? That, no doubt, is a question that will preoccupy lunar microchemists for a long time to come. This, luckily, is only part of an absorbing problem.

The flux of hydrogen atoms in the solar wind is probably about 10^8 per square centimetre per second at the surface of the Moon and the flux of carbon atoms is probably 10^4 per square centimetre per second. What this implies is that the deposition of carbon is such that six micrograms of carbon would be deposited on each square centimetre in a million years. If the inference of Cadogan *et al.* and that of similar groups elsewhere is more or less correct, the concentrations of carbon found in the lunar dust imply that the surface material has been exposed for periods of time of the order of a thousand years or more. At the same time, it is clear that the accumulation of carbon is not so great that the dust must be thought to have been exposed for millions of years. In other words, what Cadogan *et al.* have to say about methane fits in well with other observations of the properties of the lunar surface and in particular with the

evidence that the surface material in the dusty areas is frequently turned over—the garden effect, as it is called.

Where does the rest of the carbon from the solar wind reside? A treatment of this lunar dust with acids suggests that carbon atoms embedded in crystal lattices are for practical purposes the functional groups of carbides. Much of the interest in the years ahead will turn around questions such as the rate at which such carbon atoms are converted into methane groups by reaction with hydrogen. There are echoes here of the kind of chemistry now familiar in some parts of solid state physics—the effects of radiation damage, for example. But the new observations of methane and carbon in lunar dust, like earlier analyses of the isotopic composition of materials in the lunar surface, suggest important new avenues of investigation of the interaction between particles in the solar wind and the materials of which the surface is made. For one thing, the outer parts of grains of lunar dust must be eroded away by the continuing bombardment of charged particles. For another, there must be nuclear reactions between the particles of the solar wind and the atomic nuclei of the lunar material, and there will be great interest in the way in which the new observations of the lunar fines suggest that the heavier isotopes of carbon—and of sulphur, oxygen and silicon—are enriched in the lunar surface by means of what are called stripping reactions. What happens is that the lighter isotopes are preferentially removed by conversion to volatile compounds. But differentiation among the isotopes also occurs by the fractionation of repeated absorption and evaporation. To be sure, nothing in the new observations is quite as spectacular as the way in which the isotope argon-40 appears to be enriched in all kinds of materials on the lunar surface by the absorption of large quantities of the isotope released by radioactivity near the surface of the Moon in the accumulation of dust actually on the surface, but that has been a special case ever since the study of lunar samples began.

What does all this imply? To begin with, there is plainly a rich harvest to be won from further study of the microchemistry of the lunar surface. This in turn may yield a better understanding of the comparatively recent history of the lunar surface than can be obtained by other methods. In this connexion, it is especially important that the microquantities of compounds or isotopes which have now become detectable can serve as tracers to distinguish between, say, older and more recent material on the surface. But in the long run, there is also a chance that studies of the kind that have now been mounted may help to throw light on a quite different class of recent problems—that of accounting for the occurrence of comparatively exotic chemicals in interstellar space. These too are situations in which chemical compounds appear to be formed out of unlikely combinations of atoms in unlikely circumstances.

Maffei 1, and Companions

THE hitherto unknown galaxy in the Local Group that earlier this year it was surmised must be the origin of the infrared object Maffei 1 may have some near neighbours in space. One of these is Maffei 2, a second infrared object 40' from Maffei 1 the appearance of which at radio frequencies is described on page 35 of this issue of *Nature* by J. L. Caswell of CSIRO, and on page 36 by J. C. Webber and A. G. Willis of the University of Illinois. Both objects are more noticeable in the infrared than at other wavelengths because they are being seen through the gas and dust clouds of the Milky Way, which is what made Paulo Maffei report their existence in 1968. In a third related article by S. Van den Bergh of the University of Toronto, also on page 35, the galaxy IC 342, again obscured by the Milky Way and hitherto classified only as a possible member of the Local Group, is also linked with Maffei 1.

When the observations of Webber and Willis at about 2.7 GHz and of Caswell at 178 MHz are combined with observations at other frequencies already in the literature, the spectral index of Maffei 2 is seen to change in a way which is not incompatible, at least, with the behaviour of a galaxy. Webber and Willis go on to report that they have noticed a diffuse region of weak emission at 11 cm surrounding Maffei 2, and assuming the same distance of 1 Mpc which seems to be the most probable distance for Maffei 1, the diffuse source would have the linear dimensions expected for the inner regions of a spiral galaxy.

IC 342, Van den Bergh points out in the third article, is the fourth largest spiral galaxy known, and is 12° away from Maffei 1. Taking the distance of IC 342 to be 2 Mpc, this means that its projected separation from Maffei 1 has the comparatively small value, in intergalactic terms, of 0.4 Mpc. Van den Bergh goes on to remind his readers that astronomers such as Shapley, Seyfert and Hubble have already suggested that IC 342 might be in the Local Group, back in the thirties, but points out that if its distance is indeed about 2 Mpc, as the measurements seem to suggest, then it is probably too far to be what he calls a *bona fide* member.

None of this, to be sure, is in itself surprising work. In the initial article

in *Astrophysical Journal Letters* recognizing the true nature of Maffei 1, H. Spinrad *et al.* referred to the possibility that both Maffei objects are galaxies (163, L25; 1971). And IC 342 often appears in lists of members of the Local Group, albeit with a question mark beside it. But what is significant is the clearer definition of the boundaries of the Local Group that work on these objects seems to be leading towards. All members of the Local Group so far seem to be contained fairly well within 1 Mpc, and the question will be how these new galaxies, possibly at greater distances, affect the dynamics of the Local Group. Maffei 1, for example, seems to have a larger kinetic energy than any other member known so far, and it will be interesting to see how Maffei 2 and IC 342 affect the calculations.

Astronomers will therefore be looking forward to more precise determinations of the distances and masses of these three objects. All three are difficult to discern through the gas and dust of the Milky Way, and the elucidation of their nature is going to be a challenge for optical, radio, and infrared astronomers.

SEISMOLOGY

Los Angeles, 1971

from our Geophysics Correspondent

ONE of the centrepieces of the crowded schedule of the fifty-second annual meeting of the American Geophysical Union in Washington, April 12–16, was an excellent panel discussion of the big earthquake of February 9 north of Los Angeles. Big in impact, that is—the event was “only” a magnitude 6.5 earthquake of which there are usually thirty or so every year in more remote spots. The speakers at the discussion were Dr C. R. Allen (Caltech), Dr J. N. Brune (University of California, San Diego), Dr J. C. Savage (US Geological Survey), Dr G. W. Housner (Caltech), and Dr D. Tocher (Earthquake Mechanisms Laboratory, San Francisco). The following can only briefly summarize a remarkably good session, impressive for its high level of presentation and the speed with which the field work reported has been done.

The earthquake seems to have been initiated at a depth of about 13 km beneath the San Gabriel Mountains and was propagated to the surface as a rupture on a plane striking N 72 W and

dipping at about 45°. The horizontal extent of this rupture where it broke surface was 12 km and both surface information and teleseismic observations indicate that land to the north of the fault overrode that to the south of it, and moved westward simultaneously. The overriding and lateral displacement each seemed to be of the order of a metre. Sylmar is the only town through which the fault passed, and there it showed up as a spectacular shortening of streets and sidewalks. Further north, nearer the point below which the quake started, are the Veterans Administration Hospital in which more than thirty patients were killed and the Pacoima Dam (not to be confused with the Van Norman Reservoir, south of Sylmar, which posed the greatest threat).

The area is well instrumented with strong motion accelerometers and these indicated that the peak acceleration near the fault approached that of gravity. It was suggested that the value of 1 g was about the largest that would be expected from any earthquake—those of greater magnitude involved stress drops of the same size of 30–60 bars and similar near surface accelerations and the only variable was the fault length over which rupture occurs.

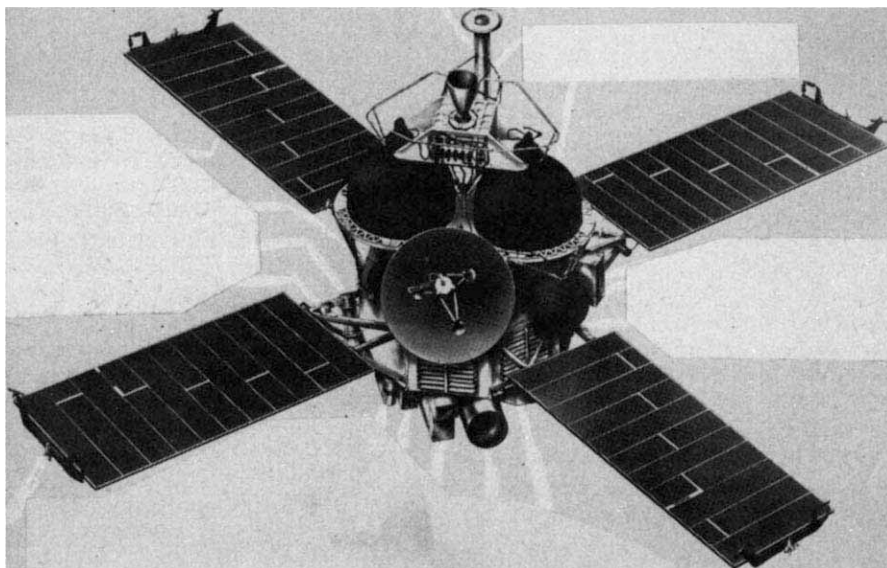
It is natural to ask how this earthquake relates to the San Andreas Fault which is only 20 km to the east. It was reported that the numerous instruments in the vicinity of that fault gave no indication at all that the San Andreas had moved during the earthquake. The inferred focal mechanism also, of course, bore no direct relationship to the characteristic right-handed strike slip faulting of the San Andreas. The explanation proposed is, however, simple. The San Andreas Fault, to be a transform fault plate boundary between the North American and Pacific Plates, should be a small circle about the pole of rotation (in Eastern Canada) between the two plates. This it is not. Although the general trend is correct, there are major bends in the fault, notably north of Los Angeles, possibly being controlled by lineaments in California that precede the initiation of the San Andreas Fault. The bend north of Los Angeles impedes uniform sliding along the fault plane and presumably the compression built up by this constriction was relieved by the overriding mechanism.

An as yet unpublicized feature of the earthquake is the extent to which building codes for earthquake resistance were successful. Nobody whose property straddles a fault break can expect to escape losses, although the speakers were at pains to point out that this particular fault had no previous seismic history and thus could hardly have

been the subject of any public warning. Nevertheless buildings nearby fared surprisingly well. Notable exceptions were two hospitals. The Veterans Administration Hospital contained three buildings, two of which were designed after the introduction of an earthquake resistant code, and these two were the buildings that were relatively unscathed whereas the third collapsed completely. The Olive View Hospital, however, close to the Veterans' Hospital and built very recently suffered substantial damage although it avoided complete collapse. It was noted that the problems still facing earthquake engineers are particularly serious in the following fields: (1) Old buildings, which include large numbers of public schools. (2) Freeway structures; a bridge whose span suddenly reduces by 1 m can hardly be expected to stay up, but bridges near the fault zone should be able to withstand the shaking. (3) Utilities; gas and water pipes. (4) The contents of buildings; the structure of many buildings remained sound in this earthquake but there was extensive damage within. Notably, an important constituent of Los Angeles' power supply from the north has been put out of action for a year by the collapse of transformers inside a substation.

SPACE

Attempt to Orbit Mars



1971 Mariner spacecraft, top view.

AFTER Mars makes one of its regular close approaches to the Earth in August, two Mariner spacecraft will be injected into orbit around the planet, if all goes well with NASA's plans. To judge by the remarkable record of the earlier close flyby experimental space-

craft, Mariners 6 and 7, the latest venture has every hope of success. These earlier craft are still operating and being used for investigations of the interplanetary medium and tests of general relativity, two years after the completion of their primary mission.

Once again, duplication will provide a partial fail-safe capability. Mariner H and Mariner I, due to be launched on May 7 and May 14 respectively, are designed to carry out different programmes of investigation, but if one satellite fails the other can provide a less detailed coverage of both programmes. The interval between the closest approaches of Mars to Earth is roughly 16 years, so that this opportunity has come at just the right moment in the development of space technology, which is building up the reliable systems which will be needed for the even rarer grand tour missions to the outer planets later this century, if they go ahead.

In order to obtain a broad picture of the Martian environment and the changes seen on the surface and in the atmosphere, one satellite will map a large part of the planet (roughly 70 per cent) while the other repeatedly studies selected areas of particular interest. The spacecraft will carry instruments for four principal experiments. The topography of the planet will be reported by both wide angle and telephoto lens TV cameras, the surface temperature will be measured with an infrared radiometer, the structure of the atmosphere and its composition will be indicated by ultraviolet spectroscopy and the composition will be determined by an infrared spectrometer. Two other experiments, the determination of atmospheric pressure and structure by

X-ray Supernova Remnant Defended

NEXT Monday's *Nature Physical Science* includes a report of further radio measurements on the supernova remnant Milne 56 made with the 64 m Parkes radio telescope by Milne and Dickel. They find that their measurements support the identification of the X-ray source GX5-1 with the supernova remnant and claim that other workers have underestimated the angular extent of the remnant in coming to the conclusion that the two are unrelated.

The new measurements have been made at 2,700 MHz and 5,000 MHz and reveal a number of interesting facts. First, the total power isotherms of Milne 56 fall away much more sharply on one side than the other and the overall shape of the contours, especially at 5,000 MHz, is the characteristic crescent expected for supernova remnants. Second, and more important, there is a considerable extension of the region of radio emission to the south at 5,000 MHz: this region includes the best known location of GX5-1 and leads Milne and Dickel to make their statement about the relationship between the two. To emphasize their argument they point out that at least six other supernova remnants are also sources of X-ray emission.

They also describe polarization

measurements in the vicinity of Milne 56. At 2,700 MHz they found that the background polarization was high but that they were unable unambiguously to attribute it to the remnant. The situation was different at 5,000 MHz, however, and a smaller background polarization revealed a significant polarization increase in the immediate vicinity of the source which follows the crescent shape and reaches a maximum just to the north of the source peak. One reason for the differences in polarization at the two frequencies could be the relative flatness of the remnant spectrum compared with the background—using both their own data and previous data, Milne and Dickel deduce that the spectrum of the remnant has an index of -0.2 . The polarization data also lead them to the conclusion that there is a background magnetic field which is approximately perpendicular to the galactic plane but which is distorted near the supernova remnant.

One consequence of the newly discovered increase in the angular diameter is that Milne's own distance and diameter estimates have to be revised. Milne and Dickel assume a new angular diameter of $30'$ which reduces the estimated distance from 3.4 kpc to 2.4 kpc and suggests a diameter of 20 pc.

occultation of the craft as they are eclipsed, and accurate determinations of the orbits of the two moons of Mars, depend only on gross effects of the near planet conditions on the radio signals from the craft, and do not require special on-board equipment.

Cautiously, the planned experiment is on a time scale of only 90 days, although there seems a very good chance that the Mariner craft will again prove reliable for much longer. If so, a contingency plan for a year's experimental work will go into operation. It is encouraging that precautions have been taken to ensure that if either mission fails no debris will fall on Mars.

ELEPHANTS

Musth and Mating

from our Animal Behaviour Correspondent

MATING is a considerable problem for male elephants, partly because of their great weight, which has led to certain structural modifications involving loss of flexibility of the body, and partly because the females live in herds from which bulls are normally excluded. In a recent study of the Asiatic elephant (*Elephas maximus maximus* L.), J. F. Eisenberg, G. M. McKay and M. R. Jainudeen (*Behaviour*, **38**, 193; 1971) show how these difficulties are overcome.

To compensate for the limited mobility of the hind limbs and the relatively inflexible sacral joint (both of which are adaptations to bearing weight), the penis of the male elephant shows a great deal of voluntary control. It can be flexed, extended and moved up and down in the vertical plane. This means that in spite of the fact that the male looks so cumbersome, mating may be accomplished very rapidly (it may often take less than thirty seconds) and the female has to bear the weight of the male for only a short time. Elephants also make use of a part of their body which is conspicuously flexible—namely, the trunk—to investigate the scent given off by another elephant.

Another barrier which a bull elephant must overcome before being able to mate is the aggressiveness of other elephants. Typically, cows are found in rather cohesive herds, dominated by the largest and oldest female, whereas bull elephants are semi-solitary for most of their lives, having been chased away from the herds at puberty. When cows and bulls meet, a male's success in courting oestrous females seems to be a function of his age and his aggressiveness; old aggressive males are the most successful both against other males and against the older cows.

Eisenberg and his colleagues suggest that a curious behavioural phenomenon

in male elephants, known as musth, may be an adaptation to the need to be aggressive to secure a mate. During musth, a male shows a dark discharge from the temporal glands and this may often be seen running down the face. Wild males have been seen rubbing these glands with their trunk and smearing the secretion on trees. This and the fact that female elephants show considerable interest in these glands suggest that they are involved in chemical communication.

Wild and captive male elephants are well known to be dangerous during this period, and it may be that musth is a rutting period in which the males temporarily become sufficiently aggressive to overcome the aggressiveness of the older cows.

RIBOSOMES

Fluctuating Complements

from our Cell Biology Correspondent

A FEW weeks ago Stanners and his colleagues reported in *Nature New Biology* (**230**, 52; 1971) that hybrid cells, obtained by fusing mouse and hamster cells, contain both mouse and hamster ribosomes. In the *Journal of Cellular Physiology* Stanners and his colleagues now report further elegant investigations of the fluctuations of ribosomal protein and RNA synthesis, and the total ribosome complements of Syrian hamster embryo fibroblasts maintained in different culture conditions.

Stanners and Becker (*J. Cell. Physiol.*,

Replication of RNA Bacteriophages

THE RNA bacteriophages are the simplest known viruses but considering the size of their genome—a single-stranded RNA of some 3,500 nucleotides coding only three proteins—they must surely be ranked among the most sophisticated. For it is clear that the replication of these minute viruses is a finely regulated process. It has been known for some time that the coat protein of R17, Q β and their relatives promotes its own synthesis by blocking the synthesis of one of the other phage proteins, the RNA replicase. And in next Wednesday's *Nature New Biology* Kolakofsky and Weissman put forward an intriguing model of the way the replicase may promote replication of the phage RNA by blocking the initiation of the synthesis of phage coat protein. Apparently the exigency of selection pressure on the slimmest of genetic resources has led to these phage proteins evolving dual functions.

The single stranded RNA molecule which forms the genome of these phages has to fulfil two functions; it must act as a messenger specifying the phage proteins and also as a template for its own replication; it is after all the "chromosome" of these particles. Once liberated from the protein coat the viral RNA in an *Escherichia coli* is first translated like any other messenger RNA. In the case of Q β , the virus Kolakofsky and Weissman have studied, the first protein made is coat protein and the second a molecule which, together with host components, forms the replicase. The third phage protein, the so-called assembly protein, is probably not made at this early stage even though it is the first of the three phage genes and precedes the coat and replicase genes in the RNA molecule.

Once replicase is made, it can compete with ribosomes for the phage RNA,

which it uses as a template for the synthesis first of a complementary RNA strand and then the resulting double stranded molecule can act as a template for the synthesis of many progeny RNA strands identical to that causing the infection. But, as Kolakofsky and Weissman realized, when ribosomes move along the infecting RNA synthesizing coat protein and replicase they are moving in the opposite direction to replicase synthesizing a complementary RNA. The two processes cannot go on together, for if they did a ribosome would collide with a replicase and neither protein nor RNA synthesis could be completed.

How is the collision path avoided? According to Kolakofsky and Weissman, once a ribosome is on a phage RNA molecule the replicase cannot dislodge it and so the enzyme cannot make a complete RNA chain. They suggest therefore that, once replicase is made, it not only binds to the end of the RNA towards which all the ribosomes are moving but it also loops this end of the RNA to an internal site close to the position at which fresh ribosomes start making more coat protein. Once this situation has been reached all the ribosomes already translating coat or replicase genes finish their proteins and fall off the RNA.

Because it is impossible for more ribosomes to get on the RNA it is rapidly cleared leaving the molecule free of any encumbrances to the replicase which can synthesize a complete complementary RNA strand, and then crank out progeny phage genomes. Later in the replication cycle the coat protein can return the compliment. By binding to a particular site on the many progeny phage RNA molecules it promotes its own synthesis by blocking the synthesis of more replicase.

77, 31; 1971) have measured the incorporation of thymidine, uridine and valine by hamster embryo fibroblasts as they progress from an exponentially dividing state to a confluent and stationary state. In stationary cells the incorporation of these three compounds is, respectively, 10 per cent, about 25 per cent and 30 per cent of that in exponentially dividing cells. The decline in the rate of protein synthesis as cells become quiescent seems to result from a combination of a decrease in the absolute number of ribosomes per cell and a decrease in the rate at which ribosomes associate with messenger RNAs and initiate protein synthesis. For the average hamster fibroblast in stationary phase has only about half the number of ribosomes present in the average cell in an exponentially growing culture, and, although almost all the ribosomes in exponentially dividing cells are in polysomes, up to about one third of the ribosomes in stationary cells are found free, not in polysomes and presumably not therefore taking part in protein synthesis.

Why is the proportion of ribosomes engaged in protein synthesis smaller in stationary cells than in dividing cells? Experiments involving the treatment of cells with cycloheximide and actinomycin indicate that this decrease in the number of polysomal ribosomes is neither the result of a shortage of messenger RNA, nor the result of a decrease in the efficiency of protein synthesis once it has been initiated. Rather, all the circumstantial evidence suggests that ribosomes in stationary cells have, for reasons that are as yet completely obscure, a lower probability of binding to messenger than have ribosomes in exponentially dividing cells. And direct measurements of the disappearance of pools of free ribosomes (induced by treating the cells with puromycin) in exponentially dividing and stationary cultures confirm this notion. The half times of the disappearing pools of free ribosomes are respectively 2.4 min and 4.4 to 5.5 min.

The ribosome complements of cells in stationary cultures, exponentially dividing cultures and synchronized cultures of cells in the G_1 phase of the cell cycle (obtained by the mitotic synchronization method) have been measured by Becker, Stanners and Kudlow (*ibid.*, 43). As a measure of ribosome numbers they assayed amounts of 28S and 18S ribosomal RNA by a polyacrylamide gel electrophoresis method, and cell number and cell volumes were measured with an electronic counter. In short they find that although stationary and G_1 cells have about the same volume, the average stationary cell has only 70 per cent the number of ribosomes present in the average cell in

G_1 , and the average ribosome complements of cells in an exponential culture and cells actually in mitosis are respectively 140 per cent and 190 per cent that of a G_1 cell.

From these results Stanners and his colleagues argue that the physiology of cells in stationary, quiescent cultures is

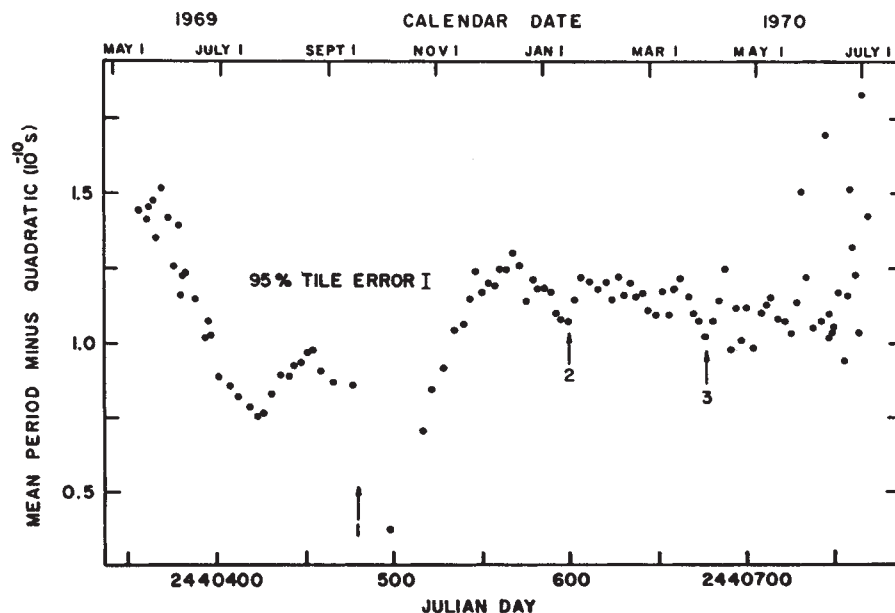
distinct from that of cells merely arrested in the G_1 phase of the cell cycle. The quiescent cell suffers from a significant deficiency in ribosomes. The intriguing question now, of course, is how the rate of synthesis of ribosomes is reduced as cells pass from a dividing to a stationary state.

Where the Crab Pulsar Went Wrong in 1969 and 1970

ASTRONOMERS with a taste for nostalgia will remember how pulsars were going to become a new time standard that would rival atomic clocks and be the basis for a new navigation system. A great deal of water has flowed under the bridge since then. Several pulsars are now known to be measurably slowing down as they run out of energy, and the regularity of two pulsars is known to be disturbed by hiccups that would be the despair of a watchmaker. Of these two pulsars, NP 0532 in the Crab Nebula is the best studied, and next Monday's *Nature Physical Science* includes an account of its behaviour

the period by about 2.3 parts in 10^9 , possibly due to the same mechanism that caused an abrupt shortening of the period of the Vela pulsar (PSR 0835-45) in February or March 1969. Obviously other fluctuations in the regularity of NP 0532 have occurred, particularly toward the end of the sequence of observations that Roberts and Richards are reporting, in June and July of last year.

The long-term running down of the pulsar on which these fluctuations are superimposed has also been closely examined by the Arecibo group. It is obviously not a linear slowing down,



Difference between the observed period and a quadratic function.

from May 1969 to July 1970, based on observations at Arecibo Radio Observatory.

The running down of NP 0532 which lengthened the approximately 33 ms period by about $1\frac{1}{2} \times 10^{-5}$ s during the fourteen month duration of the observations dominates the results, but once it has been allowed for, as it has been in the accompanying illustration taken from the article by J. A. Roberts and D. W. Richards of Arecibo Observatory, other irregular fluctuations become visible. In the illustration, for example, the event marked "1" is the period jump at the end of September 1969, which corresponded to a shortening of

and assuming that the repetition frequency of the pulses, f , follows a law of the form

$$df/dt = -Kf^n,$$

and Roberts and Richards have sought to determine the value of the exponent n . Different accounts of the way energy is lost from the pulsar predict different values for n . But the value $n=2.6$ which turned out to be the best description of the slowdown from the analysis by Roberts and Richards is not predicted by any of the current theories. But there are some strange variations from this behaviour which Roberts and Richards describe in their article.

ICHTHYOLOGY

New Fossil Teleost



A LARGE fossil teleost recovered from the Upper Cretaceous Oldman Formation in Alberta by C. M. Sternberg as long ago as 1937 has now been described by D. Bardack (*Publs. Palaeont. Nat. Mus. Canad.*, No. 3; 1970) as a new genus and species (*Paratarpon apogeronatus*). Although the specimen lacks a head, it is otherwise intact enough for Bardack to say that it is related to the living elopids and it seems to be most similar to the genus *Tarpon*. This makes the find the earliest record of this type of modern elopid in North America.

SOLIDS

Organic Semiconductors

from a Correspondent

At the Faraday Society discussion on electrical conduction in organic solids at the University of Nottingham from April 14-16, experimental work on molecular crystals, polymers, glasses and biological materials was discussed.

In the introductory lecture, Professor M. Pope (New York University) reviewed recent advances in the understanding of photoconduction and semiconduction in crystals of the anthracene type. Although these materials are highly insulating, a clear idea of band structure and the mechanism of carrier generation and migration is emerging from photoconduction studies.

Steady progress is being made in the determination of the basic data relating to conduction processes. Professor H. Akamatsu (University of Tokyo) reported measurements of work function, and several workers presented measurements of carrier mobility. Dr W. E. Williams (EMI, Hayes, Middlesex) has measured drift mobilities in rubrene by the pulse method of Professor W. Spear. Low frequency Hall measurements on 7,7,8,8-tetracyanoquinodimethane (TCNQ) salts were reported by Dr A. R. Blythe (ICI, Runcorn) and microwave Hall measurements on polymeric TCNQ salts and biological materials were reported by Professor D. D. Eley and his colleagues (University of Nottingham). The fact that mobility measurements can now be

made over a wide range of materials is encouraging, but work still has to be done to establish the limitations of the different methods and the relation between mobilities obtained in different ways.

The contribution by Professor J. M. Thomas (University College of Wales, Aberystwyth) concerning the possibility of observing proton conduction in suitable organic crystals using palladium-hydrogen electrodes sparked off some lively discussion. Professor L. Glasser (Rhodes University, South Africa) reported experiments in which imidazole was used successfully as a proton injecting electrode. In the ensuing debate on proton transfer and migration the discussion inevitably turned to conduction in ice and the recent work of von Hippel. From the interest shown it seems likely that proton conduction is likely to be one of the major growth areas in the field during the next few years.

Although the possibility of developing useful devices is clearly the motivation of many workers in the field, only one contribution was concerned with a possible application. Using a variety of electrodes, Professor M. M. Labes (Drexel Institute of Technology, Philadelphia) has observed bistable switching in thin films of aromatic hydrocarbons, in some cases with added dopants. He attributes the effect to the formation of conducting filaments.

In the biological section the most

notable advance was the ability to make measurements on ordered biological systems. Professor Eley reported measurements on mitochondria and Professor B. Rosenberg (Michigan State University) and Dr P. J. Reucroft (University of Kentucky) respectively discussed lipid bilayers and chlorophyll films. From the discussion that followed, it was clear that the conference had been highly successful in bringing together and promoting discussion between those workers interested in the fundamentals of the subject and those interested in its application to biological problems.

MOTOR VEHICLES

Exhaust Emission

from a Correspondent

A COLLOQUIUM was held at Queen Mary College, London, from April 22 to 23, to discuss the present state of knowledge of air pollution by road vehicles with particular reference to Britain's situation.

Professor M. W. Thring (Queen Mary College, London) divided the atmosphere into three critical zones. Zone 1 is the local region of closely spaced vehicles in a tunnel or street with high buildings where the time scale is 1 hour—here carbon monoxide can certainly reach unhealthy levels and diesel smoke and odours can have

Metabolic Modification of Collagen

THE principal biological function of collagen is to transmit forces or to provide a structural container for organs. It is frequently considered to be metabolically inert. That this is not so is demonstrated by two articles in next Wednesday's *Nature New Biology*. Spiro, Lucas and Rudall of Harvard University, the Shirley Institute and the University of Leeds, respectively, have studied the chemical bonding between hydroxylysine in collagen and certain sugars. The collagen they examined is of interest because it was obtained from the silk of the sawfly *Nematus rebesii*. Rudall has recently pointed out that the structure of the silk is not confined to the common β -protein fold but also exists in the α -protein and collagen conformations. Sawfly silk has already been shown to have the collagen structure. Collagens from the common fibrillar sources of vertebrates and invertebrates contain the amino-acid hydroxylysine and to varying degrees these residues are covalently linked to glucosylgalactose and galactose sugar units. The earthworm cuticle, however, contains no hydroxylysine. Spiro *et al.* have shown that the

sawfly silk collagen must be classed by itself because a high fraction (37 per cent) of its residues are hydroxylysine but none of this is bonded to sugar molecules. They speculate that the reason is that the essential enzyme glucosyltransferase is missing so that potential "carbohydration" sites remain unoccupied.

In the second article Marc Dresden of Baylor College of Medicine, Houston, presents some evidence for the part played in collagen metabolism by the enzyme collagenase discovered some ten years ago. It had been shown *in vitro* that if substrate collagen is undenatured, the effect of collagenase on tadpole tailskin is to cleave the α -chains at a specific site and to produce a fragment 75 per cent of the original molecular length. In regenerating limbs of the newt and rat uterus, fragments of 67 per cent and 62 per cent of the original length are obtained.

It is now reported that *in vivo* there exists a small fraction of the collagen in the form of fragments resulting from collagenase action on tadpole tailskin, and the 75 per cent fragment is degraded in a similar way to that in rat uterus.

unpleasant consequences. Zone 2 has a time scale of 1 week and is the valley or town where a persistent inversion traps the air; when such zones occur oxides of nitrogen and hydrocarbon vapours are serious factors. Such zones are not often critical in European conditions. Zone 3 is the whole atmosphere; carbon monoxide has a detectably higher concentration in the northern hemisphere than in the southern hemisphere because there are more cars, but it is being removed continuously in the tropopause and does not build up. There is some evidence that lead from car exhausts is building up globally and a US commission has recommended that there be no further increase in the rate of output of lead.

Dr M. J. A. Hoare (Ricardo and Co. Ltd) described the present and proposed legal restrictions in the United States and Europe and pointed out that Britain and Italy are the only European countries without legislation. The proposed European regulations restrict carbon monoxide and hydrocarbon emission, but not as severely as the present US restrictions. Dr P. Draper (consultant, formerly of Shell-Mex and BP Ltd) suggested that it should be made compulsory for all vehicles marketed in the United Kingdom to meet a revised British standard specification which stipulates a maximum smoke emission at all engine throughputs well below the just visible level. He showed how the average carbon monoxide emission per petrol engine could be reduced to an acceptable level in the United Kingdom with a better carburettor or with fuel injection equipment. Check tests which should show not more than 3.5 per cent carbon monoxide should be made by garages using relatively inexpensive CO meters under hot idling conditions. Dr G. P. Richard (Esso Research Ltd) showed how the hydrocarbon in the exhaust can reach 1 per cent on the over-run, and said that careful control of air fuel ratio and of spark timing can meet European requirements for a long time and will meet US standards until 1974. Operation with lean mixture, however, even with fuel injection will not give the very low CO and $C_n H_m$ emissions demanded in the United States by 1975, so that thermal reactors or catalytic after burners will be necessary. Recycle of exhaust gas will also prove useful for the control of nitric oxide emissions.

Dr D. Barker (BP Research) described the oxide catalysts which are liable to break up and some work on platinum catalysts was also described. Dr A. Lyon (Calor Gas Ltd) showed how far propane could solve the problems associated with carburetion of liquid fuels and also described its use at full rating for the top 30 per cent

of the fuel supply of a diesel engine to reduce smoke emission. Dr P. E. Bright (Shell Research Centre) concluded that the spark ignition engine would provide the most acceptable system up till 1980, but that the motorist might have to pay up to 50 per cent more to meet the US pollution requirements. Dr T. Weedon (British Leyland Motor Corporation) said that at the moment a very elaborate experimental system could nearly reach the 1975 test requirements for a short time only.

Dr I. M. Khan (CAV Ltd) gave a fundamental study of smoke formation in the diesel cylinder, the basic factors being the dwell time of the fuel in the over rich region and the temperature. Speeding up the mixing and using earlier injection, and water addition could all greatly reduce smoke at full power. Dr C. Young (Ford Motor Co.

Ltd) described the very extensive laboratory being built at Dunton to carry out 20,000 emission tests on cars a year under European and American conditions. Dr G. Rice (University of Reading) said that the Sterling engine was the only one now capable of meeting the standards proposed for the United States in 1980 and Dr J. W. Todd (Texaco Ltd) described a very interesting stratified charge engine which can give 20 per cent fuel saving, use any fuel (so no lead is necessary) and has very complete combustion with low NO_x .

Professor Thring concluded that Britain can achieve a reasonable compromise by instituting tests to ensure (a) that in spark ignition engines the ignition timing and carburettor performance are of a high standard, and (b) that in diesels, there is good mixing and fuel injection timing.

Significance of Ophiolites

ALTHOUGH, thanks largely to palaeomagnetism, a great deal is now known about the large scale evolution of the Earth's surface over the past 200 million years or so (that is, since the onset of continental drift in the Wegenerian sense), knowledge of pre-Triassic activity on the continental and oceanic scale is rather more shaky. The reason for this is obvious, of course—the mists of time become thicker with age; but the point is worth making if only to counterbalance the confidence inspired by the successes of the past decade in tracing the progress of continental drift and sea floor spreading during the Mesozoic and Cainozoic. The fact is that very little is known about the configurations of continents and oceans during the Palaeozoic, let alone the Precambrian; and what evidence there is often seems to be conflicting.

The evolution of the Appalachian-Caledonian Orogen is a good case in point. The evidence is such that it has been possible to formulate several different models for the origin and history of this system; but as so often happens in geology no firm decision can be made in favour of any of them. In next Monday's *Nature Physical Science*, however, Bird *et al.* point to at least one important property of the Appalachian-Caledonian system—the existence of ophiolites—which they claim points some way towards its origin. As Dewey *et al.* (*J. Geophys. Res.*, **75**, 2625; 1970) concluded some time ago, there is evidence to suggest that ophiolite complexes were originally oceanic crust and mantle generated by accretion at plate margins (oceanic ridges) and in marginal oceanic basins.

If this is so it clearly becomes important to look closely at what ophiolite sequences exist within the Appalachian-Caledonian system. Accordingly Bird and his colleagues have compiled a list of basic and ultrabasic rocks in this system which they believe to belong to the Appalachian-Caledonian ophiolite suite.

Most of the ophiolites that Bird *et al.* list are known, or suspected, to be Ordovician except for two examples in Newfoundland which must be older and are, in fact, probably Cambrian. The fact that Ordovician ophiolites preponderate has apparently led some workers to suppose that the proto-Atlantic Ocean (a former ocean which existed within the area of the present Appalachian-Caledonian Orogen and which coincided roughly with the position of the present central and north Atlantic but which had completely closed by the end of the Devonian) originated during the early Ordovician. With this interpretation Bird and his colleagues disagree. They adduce evidence, for example, which requires the existence of an ocean during the Cambrian and which implies the presence of continental margins during the late Cambrian-early Ordovician Grampian orogeny. Moreover, the older ophiolites in Newfoundland suggest some proto-Atlantic sea floor spreading during the Cambrian. In an alternative interpretation Bird *et al.* conclude, with evidence, that the proto-Atlantic Ocean actually began to grow during the late Precambrian. This is then consistent with the supposition that the early Ordovician Appalachian-Caledonian ophiolites were the result of lower Palaeozoic sea floor spreading.

CHROMATIN

Nuclear RNA Nobbled

from our Molecular Biology Correspondent

THE silence concerning the nature of Bonner's chromatin RNA has now become nothing less than deafening. It was alleged to be a defined species, some forty nucleotides in length, more than a quarter of which were dihydro-uracil. It was present in plant and animal nuclei, showed a very high degree of hybridization with the DNA, and its implication in control of transcription was boldly inferred. Heyden and Zachau (*Biochim. Biophys. Acta*, **232**, 651; 1971) have now carried out a painstaking examination of the nature of the RNA component of chromatin and the procedures used for its preparation, with dispiriting results.

Starting from calf thymus, and adhering closely to the procedure described by Shih and Bonner, Heyden and Zachau quickly encountered their first problem; the nucleoprotein pellicle—the term reserved for the scum that floats to the surface in caesium chloride centrifugation—has to be treated with pronase to liberate the nucleic acids. Pronase, being essentially a crude extract of fungus, contains a fearsome nuclease activity, which falls only during incubation, when the proteases gradually consume the nuclease components (as well as each other). Conditions were, however, found which led to the elution of two nucleic acid peaks from an ion-exchange column, as described by Shih and Bonner. One of these was DNA, and the other was identified as RNA, by the device of treating the eluted fractions with tritiated sodium borohydride. This effects the reduction of the dihydrouracil ring to an open-chain alcohol, with introduction of tritium in sufficient quantity for counting. Before column fractionation this method failed, the RNA being evidently protected, perhaps by protein fragments.

The RNA column fractions showed in polyacrylamide gel electrophoresis only a broad smear of low-molecular weight material. If the pronase treatment of the pellicle was omitted, however, the RNA showed instead a major component migrating in the position associated with tRNA. The identification was confirmed by the demonstration in the extract of high amino-acid acceptor activity, which was substantially destroyed by the pronase treatment. Indeed, treatment of samples of tRNA with pronase under the standard conditions of the chromatin RNA preparation led to a low level of activity and a smear of degraded material exactly like that resulting from the procedure of Shih and Bonner. Gritting their teeth, Heyden and Zachau then

showed that with effort a largely nuclease-free pronase could be prepared, and its use led to the isolation of good tRNA from the chromatin; largely undergraded tRNA could also be extracted from the pellicle by treatment with urea.

They go remorselessly on to show that the endogenous tRNA hybridizes to a degree of 0.08 per cent, with its DNA, but if it is treated with crude pronase, so as to cause moderate degradation, the saturation level is increased by a factor of three, which

brings it into the range achieved with the chromatin RNA fraction, though not, it must be owned, with the enormous value of 5 per cent, reported briefly by Dahmus and Bonner (*Fed. Proc.*, **29**, 1255; 1970) for preparations from some other sources. There seems then little room for doubt that the bulk of chromatin RNA preparations is tRNA. That some small proportion is a chromatin-specific species cannot of course be excluded, but anybody seeking to demonstrate that this is so must evidently start from scratch.

Translational Control by Sex

THE well established control of gene activity in bacteria operates at the level of transcription. Repressors and RNA polymerase factors regulate different genes according to the temporary requirements of the cell. But do bacteria also regulate the level of translation of different messenger RNAs? That is, can cells choose to translate specific classes of messenger RNA molecules and to reject others? It seems that infecting bacteriophages do use such controls to appropriate the ribosomes of bacterial cells: Hsu and Weiss (*Proc. US Nat. Acad. Sci.*, **64**, 345; 1969) showed that an *in vitro* protein synthesizing system from bacteria infected by bacteriophage T4 is more receptive to T4 messenger RNA than to *Escherichia coli* messenger RNA or RNA isolated from RNA phages. This preference occurs at the level of binding of ribosomes to mRNA (Dube and Rudland, *Nature*, **226**, 821; 1970), suggesting a control of the initiation of protein synthesis. Steitz *et al.* (*Nature*, **226**, 824; 1970) also showed that the residual binding by ribosomes from T4 infected cells to RNA phage messenger displays a considerable change in the relative preference for different initiation sites on the RNA molecule. These effects of altered binding reside in a high salt wash of ribosomes; because this is known to contain the initiation factors, Steitz *et al.* suggested that altered binding may reflect a replacement of *E. coli* initiation factors by new factors specific for initiation sites of T4 mRNA.

In next Wednesday's *Nature New Biology*, Morrison and Malamy make similar arguments from quite different experiments with phage T7. These authors show that phage T7 cannot grow in male *E. coli*, that is bacteria which contain an episome (a piece of autonomous DNA) called an F factor, because most T7 messenger RNA is not translated into protein in male cells. Female cells, which lack the F factor, do support the growth of T7. Although both early and late classes of T7 messenger RNA are synthesized in male cells, only the very earliest class

of T7 proteins is found. Thus the machinery of protein synthesis in male bacteria can distinguish different classes of mRNA, an ability which would be fundamental to a translational control mechanism.

How do male cells manage this discrimination? In order to approach this question, Morrison and Malamy isolated mutants of male bacteria which do allow T7 to grow. As expected, these mutations map in the F factor. Apparently two F factor mutations (PIF⁻ 2A and PIF⁻ 2B) must be present for T7 to grow in the male cell; if only PIF⁻ 2A is present, some, but not all, late T7 proteins are made and no progeny phage are produced, although the host cell is lysed. The PIF⁻ 2B mutation has not been isolated alone. Thus the F factor seems to encode two proteins which together completely prevent the translation of late T7 mRNA.

Morrison and Malamy cannot yet say what these proteins do, although they are in favour of the interpretation that these are specific initiation factors for F factor mRNA which compete against initiation factors made by T7 specific for late T7 mRNA, but which do not compete against the normal initiation factors which serve *E. coli* mRNA and T7 early mRNA. If T7 does make specific initiation factors, it seems surprising that no conditional lethal mutants of these have been found; Morrison and Malamy argue, however, that those mutant selection procedures which have been used would not have detected them.

Although these and previous experiments suggest strongly that translation of certain classes of mRNA can be prevented, they do not yet establish a general mechanism of translational control through availability of different specific initiation factors. It is still possible, for example, that F factors and phage T4 simply produce proteins which bind to T7 and *E. coli* mRNA and thereby prevent their interaction with ribosomes. Morrison and Malamy seem to have a good system with which to provide an answer to this problem.

Design Education in Great Britain

MISHA BLACK

Design Research Unit, 32 Aybrook Street, London W1M 4BB

The concept that art and design education are indivisible is being eroded, and new concepts of industrial design are emerging. Professor Black argues for specialization in art and design education and for specialist design courses to be given in the schools of art.

THERE are some 26,500 students taking full time courses at art schools in England and Wales and, if part-time and evening students are added, the total is more than 111,800. About 22,700 of these students are studying art or design in courses leading to recognized qualifications and in preparation, they hope, for a socially useful and personally satisfying career¹. Considered in isolation, this figure is startling—it implies some 6,000 to 7,000 young graduate artists and designers seeking new employment every year—but measured against the some 400,000 full time science and humanities students in the universities, polytechnics and other institutions of higher education the turnout of the art and design colleges is minimal. It can be argued that there is a need for society to be injected each year by this relatively small number of art and design graduates, yet there is little doubt that a large proportion of the graduates from art colleges do not find acceptable employment and are frustrated by the knowledge that they are unwanted men and women doomed to jettison their professional potentialities if they are to earn a livelihood.

It is easy to argue that the number of art school students should be reduced to equate them more directly with job potentialities, that art and design colleges should be reduced in number, that professional training should be more directly vocational and that Britain should be supplied with only those artists and designers for which a clear need exists. But this is a counsel of despair; curtailment of art and design education would be the action of an emasculated society which is only able to visualize its future in the misleading terms of simple arithmetic. It is not the size of the provision that is wrong, it is the antiquated concept of art and design education which needs to be modified.

The original design schools of the mid nineteenth century were specifically geared to produce art technicians. Painting and drawing were taught as tools of a trade, connexion with the fine arts was discouraged. Initially drawing from the nude

was forbidden as being unnecessary and overstimulating. But the dichotomy between design and art could not be long sustained in Victorian society which believed that mass produced embossed brass fire screens were artistic. By the mid-1930s the process had been completed. A few schools of art and crafts paid lip service to the needs of industry and of "commercial art"; none in Britain provided an effective education in design.

The deep rooted assumption that art and design are one was not easily disturbed. The first report of the National Advisory Council on Art Education published in 1960² accepted the seminal importance of the fine arts: "... fine art teaching in a school—whatever form the fine art takes—can serve, as we believe it should, as a focal point of strength and inspiration for the whole school . . . it is through this teaching (of the fine arts) that students may learn something of those fundamental skills and disciplines which underlie and sustain any form of specialization in art and design".

The Monolith Tumbles

Only last year in the Report of the National Advisory Council on Art Education (NACAE)/National Council for Diplomas in Art and Design (NCDAD) Joint Committee on the Structure of Art and Design Education¹ has this official view been superseded; now these two bodies which advise the British Government on art and design education and control the examination procedures have stated "The study of fine art is not now regarded as necessarily central to all diploma studies in the design field". The monolithic art-based concept of design education has tumbled and it has become possible to contemplate how a new edifice can be constructed from its fragments.

The NACAE/NCDAD joint committee report recommends three types of art and design education: three year courses at university level leading to a general diploma in art and design (the existing but liberalized Diploma in Art and Design—DipAD); specialized design courses, also at university level, lasting four years (including a period of three to twelve months in industry) which may be attested by a different diploma; design technicians courses lasting two or three years aiming to produce qualified design assistants. Foundation studies before entry to the university level courses are maintained, and the report also emphasizes the essential role of the schools of art and design in general and adult education.

The joint committee was begotten by the student protests at Hornsey and Guildford in 1968–69 and by dissatisfaction with their curriculum more staidly expressed by students at other colleges of art and design throughout Great Britain, but

the need fundamentally to review the structure of art and design education was already apparent to all who were involved in it. The student rebellions were the spark necessary to flare conviction into action.

Ten Year Switch

During the ten years between the analysis which resulted in the first NACAE report and the student protest, three fundamental changes had occurred. First, the aristocratic concept of the fine arts had been disturbed by the emergence of art forms which were by description "popular", by the young artists' wish to produce an art in which twentieth century man could participate and which was not restricted to a self appointed elite of aesthetes. Second, those industries which had been based on craft techniques, such as textiles, ceramics and furniture, became progressively automated and demanded technical knowledge and skills from their designers which were not satisfied by the craftsman's personal inventiveness and manual dexterity. Third, instructions in industrial design (engineering) had become a new element in design education. Other influences were the establishment of departments of general studies in art schools which expanded the students' intellectual horizons and the developing interest in film and television production with their direct social connotation. The students (and some of their tutors) saw clearly that traditional art and craft education did not fit the new situation and they endeavoured to sift out new patterns which would produce graduates to match the emerging condition of art and design. Critical self examination, talk-ins and conferences cumulated in the following six basic theorems. (1) There is no dividing line between the so-called fine arts and design, and compartmentalized education is therefore irrelevant. (2) Studies should be based on network curricula allowing students to study those subjects which they themselves decide best suit their development. (3) Specialization is undesirable during this period of accelerating technological development, as techniques studied at school will be out-dated before the student can apply them. (4) The aim of art and design education should be to produce generalists and not specialists. (5) All general education qualifications should be eliminated as a requirement for entry to an art college. (6) Education is an activity shared by staff and students and the students' point of view should be expressed whenever decisions are made which affect the academic or administrative life of a college.

The militant students were, excepting at the power keg-points of Hornsey and Guildford, in a small minority. The majority were content to use their few years at college or university in learning and in the self-development of skills and personality, but it is nevertheless probable that the proselytizing students had, and still have, at least the passive support of the majority. Majority support certainly does exist for student participation in academic and administrative decision making in Britain. In the colleges of art and design, students are now commonly represented on academic boards and councils, and the result of their willingness to share in the tedium (and occasional excitement) of committee work has produced a new educational environment in which radical development is a possibility.

Art by Design

If dialogue is now permitted the students must allow the other five contentions of their manifesto to be subjected to analysis. First, the deeply felt view of many students that art and design are indivisible, that they are two aspects of the same activity. This, stated as a universal generalization, is, I believe, a misconception which arises from a confusion between peaks and plateaux. At their extremities of maximum achievement art and design are different activities sharing only creativity and some techniques in common. Art I believe to be expressive of the human condition; it provides clues to what cannot be explained in rational terms, it is concerned

with love and hatred, ecstasy and misery, life and death; it can achieve what Mondrian has called the "union of the individual with the universe"³. Design is a problem-solving activity concerned with invention and with formal relationships, with the elegant solutions to problems which are at least partially definable in terms of day to day practicability.

Students see the solution in a network organization of courses which will allow them to move from painting to design, from technical to expressive experiment as they wish. This, they argue, will provide a broad base of general experience within the boundary of visual communication which will allow them later to decide how they individually can best make their own contribution to social need. The art student will, they argue, "use this network structure to provide himself with a broad-based education free from pressure to specialize in specific academic areas. The structure allows the student to adjust his concentration in areas of study in relation to his vocational needs"⁴. This is a persuasive argument which may be valid at junior and undergraduate level even if it does produce new problems of academic organization. The danger that exists, however, in this proposed network system is in the risk of its inculcating in the student a grasshopper attitude which may inhibit later specialization. The concept of undergraduate courses in which students move freely from the expressive arts of painting and sculpture to the more constraining discipline of design is valid only if it leads to specialized post-graduate studies or, alternatively, to the acceptance of a visual arts education as being unvocational in the sense that reading history or English for a university degree is only rarely intended to provide the basis for later specialization in history or English. Many protagonists of the view that network courses are justified by the need for generalists rather than specialists do not visualize network studies as a basis for later specialization but instead as a base for continued generalization. This Leonardo syndrome is, I believe, misconceived.

Generalist or Dilettante

The protagonists for generalization argue that technology now develops so quickly that anything the student may learn will be out-dated by the time he is in professional practice. I agree with the prognostication but the educational system which the students propose would, I believe, produce the opposite to its aim. The students would presumably study design methodology and move easily from this to painting and sculpture, from visual/audio experimentation to engineering drawing, from an interest in textile design to a concern with industrialized building and computer programming. The art and design colleges would produce the new late twentieth century Renaissance man. But is it possible to achieve this end even if the curriculum and internal organization of art and design colleges are radically changed? It might theoretically be feasible if the diploma course were lengthened to an obligatory five years from the age of eighteen, but this will not be achieved within the present British pattern of advanced education. If the accepted three year limit to university education continues (as it will within the foreseeable future) with no more than 20% of the graduates continuing to post-graduate study, then a network system purporting to produce generalists can only produce dilettantes—artist/designers without sufficient technical proficiency to support their presumptions. Only the geniuses could survive so profligate an educational system.

The contrary argument is that generalization can best develop from specialization, that only those who have experienced the rigours of applying knowledge and experience creatively to the resolving of a precise task can appreciate the reality of problem solution. It is significant that those who most vigorously support generalization before specialization are always students or academic theoreticians who have never been faced with the need to focus their creativity and inventiveness to the actual solution of a problem in art or design. The

practising artist and designer know that they can only produce valid results by the intense concentration of all their professional skills on the task which they have set themselves or which, in the case of a designer, has more usually been presented to them for solution. Those who have been faced with the need to make a positive concrete creative statement know that this demands not only generalized appreciation but also a background of accumulated specialized skills and experience. If the student has not obtained at least rudimentary experience in concentrated application, in the marshalling of such technology as he has learned to the solution of a specific problem, the chances of his being able to do this in a real life situation are jeopardized. Generalized studies there must be, social awareness is essential, but the core of undergraduate studies must remain, I believe, intense concentration on known techniques even if, when studying them, the student is aware of their transient value. Art or design studies which are based largely on theory and open-ended experiment neglect the essence of the subject: the cultivation of creative skills concentrated on specific concrete problems from concept to completion and valuation.

From the central core of specialization, general and wider studies can and should be undertaken, but specialization must, I believe, initially be the control mechanism of the creative process. From specialization, design can expand to generalization; without specialization the young designer is at the mercy of the technically competent and experienced, with whom he cannot even communicate. I believe there is a special role in society for those who are educated at undergraduate level to be generalists, but generalization alone, without initial specialization, is no more connected with design than hospital management is with surgery, or railway administration with the design of locomotives.

Engineering Industries

In the light and heavy engineering industries the break with craft concepts is absolute; machines are able to produce artefacts which a single craftsman could not make however skilled he might be. The difference is qualitative and not quantitative. The relationship between designer and machine is intellectual; drawing and model making are purely a method of communication; the manual skills of the designer are eliminated. It is this revolutionary divorce between human capacity and machine capacity which has sparked much of the present controversy and re-examination. In Great Britain the number of students studying industrial design (engineering) as their main subject is small: not more than 350 at the eight schools which provide courses in this subject. ID(E) students are only some 2% of the total of those taking full time courses in art or design but the influence of this small student group is disproportionate to its size as the growth and technological development of the engineering industries indicate the future pattern of all industrial production.

So far the British schools have accepted two main streams of industrial design: that based on methodical analysis and that concerned primarily with styling, with the application of style to the staid products of the engineering workshops. While one student group is working on the rationalization of hospital equipment another may be concerned, with equal creative intensity, with the design of juke boxes or automobile bodies.

The move away from the stylistic approach to industrial design is now endemic in the schools. A new social consciousness makes the students question the role of design in marketing and the ethical validity of design for planned short term obsolescence. From these reasonable attitudes it is a short step to antagonism towards a conscious aesthetic attitude to the products of the engineering industries. The angst is the most acute in the United States where Pulos writes⁵: "The American designer seems to be caught up in the general shift from an industrial economy dependent upon the proliferation of objects with little responsibility for their effect on society to

an awakening realization that runaway affluence can be as debilitating as poverty".

The problem of desperate poverty in the midst of affluence faces all industrially developed countries, and the student industrial designers throughout the world share these misgivings. Understandable though these socially conscious attitudes to society are, the simple fact remains that artefacts are inevitably the mirror of a civilization. Style is an essential inescapable aspect of design which will always affect the engineering solutions either consciously or unconsciously; a conscious decision to discard a stylistic approach in itself produces a style. Style, and its fringe of fashion, is an aspect of all but mathematically determined engineering design; the focus of discussion is whether stylistic formality in engineering design should be consciously developed or whether it is an inevitable spin-off from technological development and therefore does not require organized education.

The products of the majority of engineering designers indicate that the method of their education fails to release appreciation of formal, ergonomic and communicative qualities. Much of engineering training is additive and inhibits later conceptual development and thus acts as a constraint on creativity and invention. This was confirmed by the Fielden report of 1963⁶ and has so concerned the Institute of Mechanical Engineers as to have moved them to set up in 1967 a committee under the chairmanship of their then president, Mr H. G. Conway, to study the problem of creativity in engineering. This committee recently reported on a visit to colleges and universities in the United States⁷ where serious attempts are being made to identify creativity in engineering design and reform curricula to encourage its development in undergraduate education.

The academic attitudes of engineering faculties in many American universities do not differ greatly from those of schools of industrial design such as the now sadly defunct Hochschule für Gestaltung at Ulm, the School of Industrial Design at the Illinois Institute of Technology, or the Department of Industrial Design (Engineering) at the Royal College of Art in London. It can therefore be rightly questioned whether development should be towards modifying the curricula of advanced level courses in engineering or towards achieving greater sophistication in courses of industrial design at art and design colleges. For the immediate future both developments are necessary. It will take many years before any engineering degree course in Britain will be modified sufficiently to replace the need for specialized industrial design education, and then, in university and polytechnic engineering courses, it will almost certainly be necessary for the special skills of industrial design to be acquired in postgraduate study.

In the meantime the industrial design departments of art and design colleges have the advantage of an environment in which the need for creativity is the norm rather than the exception, in which the use of drawing as a means of self discovery and communication is readily acquired, and where problems of style and of the relationship between man and the machine are normal subjects. I doubt whether it would be useful for a country without an already effective course in industrial design to attach a new department of industrial design (engineering) to an existing art/craft/design college, but where such courses are firmly established it would be folly to jettison them for an as yet rudimentary understanding among professors and tutors of engineering of educative techniques for releasing creativity.

Art as Education

The provision for studying art/design in Britain is too meagre rather than too ample, but it is nevertheless probably true that the present schools produce more artists and designers than Britain is prepared usefully to employ. In spite of the increasing number of art dealers' galleries, only a small minority of painting and sculpture graduates make a living from the practice of their art; some 40% of them become teachers in

general education and the remaining 60% either teach part-time in art and design colleges or take other jobs where their creative capacities may not be fully engaged. The fate of students of design is largely unknown but it is likely that a considerable percentage of the graduates do not find employment in which their specialized skills are used and developed. In August 1969 the Government Social Survey was commissioned to investigate the employment of those who have completed courses in art and design, and its report is awaited. It is probable that there is a total over-provision of places for art and design students even if readjustment of numbers is necessary to balance scarcities in some fields with excess in others. I have little doubt that, if considered purely numerically, even taking into account the essential wastage, too many students of art and design are being educated in Britain to meet even the most optimistic view of progressively greater public interest in the fine arts and of expanding opportunities for employment in industry. If the Social Survey Report confirms my prediction it is clear that many art/design schools in Britain will need to turn into centres for adult education and recreation, and fulfil a general social rather than a supposedly technical/professional role. The need for creative leisure facilities is so great that it makes the number of such centres, which a reassessment of art and design education would provide, too few rather than too many.

But the possibility remains for a new concept of the function of art and design education to exist collaterally with its professional intention. University education in the humanities is based on language, literature and the intellect; visual and

tactile sensitivity are almost completely ignored. Marshall McLuhan has argued persuasively that we are suffering from an amputation of the senses of sight, touch and hearing, while the need to re-unite the senses with the intellect is crucial as we develop audio/visual/tactile methods of communication in the midst of the present electronic revolution. University education based on visual/audio insight and sensibility could prepare young men and women for a role in society which is now largely neglected. The disciplines of art and design could provide the framework for education which does not sacrifice the senses at the altar of the intellect. These graduates would not expect to become professional artists or designers; they would be prepared to work as managers and organizers, as entrepreneurs and civil servants, but they might well become the supra-artists, not dedicated to the canalization of their talents and understanding within the confines of specialization but able to bring an artist's insight and sensitivity to focus on problems of human relations and the human environment.

¹ *The Structure of Art and Design Education*, Report of the Joint Committee of the NACAE and the NCDAD (HMSO, 1970).

² *First Report of the National Advisory Council on Art Education* (HMSO, 1960).

³ Mondrian, P., *Plastic Art and Pure Art* (Wittenborn Schultz, 1945).

⁴ *The Hornsey Affair* (Penguin Books, 1969).

⁵ Pulos, J., *Stop the World—We Want to Get On* (Journal of the Industrial Designers Society of America, September 1969).

⁶ "Engineering Design", Report of a Committee appointed by the Council of Scientific and Industrial Research (HMSO, 1963).

⁷ *Report on a Visit to America to discuss Creativity in Engineering* (Institute of Mechanical Engineers, London, 1968).

Biochemical Research in Schizophrenia

ALAN A. BOULTON

Psychiatric Research Unit, University Hospital, Saskatoon, Saskatchewan

Schizophrenia is still a loosely defined and little understood group of diseases. But a great deal of information is accumulating about the biochemical abnormalities which might be characteristic of so-called schizophrenics.

ATTEMPTS to implicate biochemistry in schizophrenia have produced a considerable flurry of thought and activity in the past ten to fifteen years. This has been concerned mostly with so-called model psychoses on the one hand, and analysis of body fluids (usually urine) on the other. Most attempts at analysis of body fluids have been motivated by interest in the model psychoses. It would be pleasing to be able to report that all this activity had produced tangible results and greater understanding. Regrettably, notwithstanding the controversies, progress has been slow if indeed there has been any. To a biochemist it becomes apparent from the literature that schizophrenia is a difficult term; psychiatrists do not agree on a precise meaning and diag-

nosis for schizophrenia. A schizophrenic population certainly seems to be a mixed bag containing possibly several separate disease entities overlapping to some extent; their adequate separation and description are difficult because of inexact diagnostic criteria. This makes biochemical research much more difficult than in more definable conditions such as Parkinson's disease or certain inborn errors of metabolism.

Nonetheless, schizophrenia, whatever it is in exact terms, is indisputably a tragic debilitating condition affecting large numbers of people in all cultures. It continues to be urgent that we attempt to obtain a better understanding of the manifestations of, and the processes leading to, the condition. This must be attempted in biochemistry as well as the other branches of medicine even though some psychiatrists still do not believe that biochemical malfunction is involved.

It would be encouraging if an obvious inheritance of schizophrenia were visible. Studies so far¹⁻⁶ indicate that while there is probably some underlying genetic component it is not clear cut and cannot be simply explained (see reviews in refs. 7 and 8). The description by Huxley *et al.*⁹ of schizophrenia as a genetic morphism is appealing, but must at this time be considered premature; as such it has been criticized¹⁰⁻¹².

In this review it is not possible to cover the vast current literature and so only the two areas where activity has recently been greatest have been selected for detailed comment.

Phenylethylamines

In 1952 Osmond and Smythies¹³ drew attention to similarities between the structure of mescaline (an hallucinogen) and the catecholamines. Harley-Mason¹⁴ predicted that an abnormal methylation of dopamine and/or noradrenaline could produce 3,4-dimethoxyphenylethylamine (DMPE) or 3,4-dimethoxyphenylethanolamine. Both these substances are chemically similar to mescaline (Fig. 1).

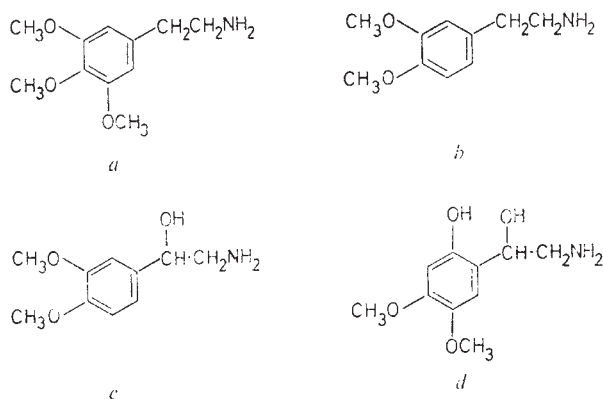


Fig. 1 Some methylated phenylethylamines. *a*, 3,4,5-Trimethoxyphenylethylamine (mescaline); *b*, 3,4-dimethoxyphenylethylamine; *c*, 3,4-dimethoxyphenylethanolamine; *d*, 2-hydroxy-4,5-dimethoxyphenylethanolamine.

This concept gained credence following the observation that feeding methionine, a methyl group donor substance, as well as monoamineoxidase inhibitors could in some cases exacerbate psychotic symptoms¹⁵⁻¹⁶. Although these results have been supported by several groups¹⁷⁻²⁰, one using betaine instead of methionine²⁰, it is very difficult to be sure that behavioural changes observed were an exacerbation of schizophrenia, for normal individuals on a similar regimen exhibit marked behavioural changes. Furthermore, this effect is not limited to methionine: similar results are obtained with tryptophan. Sprince's group²¹ has shown, in the rat, that the metabolism of these two amino-acids is closely related; increased methionine inhibits the nicotinic acid pathway and increases the excretion of indole-acetic acid and other indoles, while the excretion of N-methylnicotinamide is diminished. A rather controversial chemotherapy^{16,22,23} for schizophrenia—the ingestion of large quantities of nicotinic acid or nicotinamide—seems to be relevant here, for these vitamins are methyl group acceptors. In a double blind trial²³ the length of stay in hospital of a group of schizophrenics ingesting nicotinamide was significantly shorter than the control group.

Although N and O-methylation are recognized as general and important metabolic processes²⁴, there is no direct evidence from methyl donor feeding experiments that abnormal or excessive methylation occurs; in fact the little evidence available suggests an altered indole metabolism and involvement of certain members of the vitamin B complex.

The Pink Spot

In 1962 Friedhoff and van Winkle²⁵ reported that they had identified in schizophrenic urine β -3,4-dimethoxyphenylethylamine (DMPE), one of the amines predicted¹⁴. The extraction procedure was crude, involving simple chloroform extraction of urine first at pH 2.0 and then pH 9.0; the alkaline extract after drying was separated unidimen-

sionally on paper chromatograms in the conventional all-purpose solvent system butanol-acetic acid-water. After drying, the developed chromatograms were treated sequentially with ninhydrin-pyridine reagent and then Ehrlich's *p*-dimethylaminobenzaldehyde reagent. At an R_F position of about 0.6 a pink spot zone developed (Fig. 2). It is important to note that if DMPE is responsible for this pink spot zone, using the techniques described, at least 50 μ g would need to be present in a 24 h urine specimen²⁶. Several groups have reported that urine, both schizophrenic and normal, processed in this and alternative ways, yields a pink spot zone similar to that described by Friedhoff and van Winkle. Takesada *et al.*^{27,28} using two dimensional chromatographic techniques showed that many amines nearly isographic with DMPE as well as DMPE itself are excreted in both normal and schizophrenic urines. They did not positively identify any of the excreted amines. Von Studnitz and Nyman²⁹ claimed an exogenous origin for DMPE on the basis of its disappearance from urine when a glucose-citric acid diet was given; in view of subsequent work (for example, refs. 37 and 38), however, it seems likely that they were not measuring DMPE at all. Several groups using fairly sophisticated thin-layer chromatographic techniques³⁰⁻³² and sensitive ion exchange techniques^{33,34} failed to locate DMPE in urine obtained from schizophrenics and from a case of periodic catatonia respectively. Sen and McGeer³⁵, using gas chromatographic techniques, found between 5 and 20 μ g of DMPE and 4-methoxyphenylethylamine (MPE) in the urines of about half their schizophrenic patients. But after considerable effort this group³⁶, using numerous paper, thin-layer and gas chromatographic systems, found no evidence for significant quantities of either DMPE or MPE (that is <5 μ g/24 h) or their respective principal acid metabolites (<10 μ g/24 h) in either normal or schizophrenic urine samples. They did show that numerous other substances and artefacts could and did produce false indications that those substances were present.

Perry *et al.*^{37,38} have shown that in their schizophrenic urine extracts, DMPE and MPE, if present at all, must be less than 2 μ g/g of creatinine. They also identified two of three substances that are chromatographically indistinguishable from DMPE. They are monoacetyl cadaverine, monopropionyl cadaverine and quite probably the third is another monoacylated cadaverine, all of which seem to arise from the gut bacteria.

DMPE in Urines

In their first publication Kuehl *et al.*³⁹ described a substance isographic with DMPE that was excreted in much smaller amounts (3–10 μ g/g of creatinine) than previously reported (>50 μ g/day): the substance seemed to be unique to schizophrenia. This group later investigated the urinary excretion of 3,4-dimethoxyphenylacetic acid (DMPAA), the principal metabolite of DMPE⁴⁰; they found by unambiguous techniques (gas and thin-layer chromatography and mass spectroscopy) that DMPAA is excreted (<30 μ g/day) without significant difference between normals and schizophrenics. Felton and I²⁶ and Bell and Somerville⁴¹, using paper chromatographic and paper electrophoretic techniques and a relatively specific fluorescence procedure, found no evidence for DMPE (that is not more than 5 μ g/24 h) although a pink spot was demonstrated. Using the same techniques Felton and I⁴² showed that the pink spot excreted in Parkinson's disease, first reported by Barbeau *et al.*⁴³, also did not contain significant quantities of DMPE. We⁴⁴ identified by ion exchange chromatography, infrared and mass spectroscopy the pink spot in Parkinson and schizophrenic patients as *p*-tyramine. The excretion of this compound, which is a normal metabolite, is significantly elevated above normal in 50 per cent of Parkinson patients and about 10 per cent of schizophrenics (my unpublished

results). The significance, if any, of this fact is not as yet understood. Rinne and Sonninen⁴⁵ using sensitive and fairly specific techniques have shown that the urinary excretion of DMPE in Parkinsonians and normal individuals is not significantly different (range 4.4–6.8 $\mu\text{g}/24\text{ h}$). DMPE has now been unambiguously identified (mass spectroscopy of the Dansyl derivative) in the urine of two female schizophrenics by Creveling and Daly⁴⁶: excretion was in the range 4 to 10 $\mu\text{g}/\text{g}$ of creatinine. Unfortunately these authors did not investigate urine samples from normal individuals.

It has been pointed out^{47,48} that the metabolites of the drugs consumed by schizophrenic patients have a direct bearing on the presence of pink spots in the DMPE zone of chromatograms. Chlorpromazine, one of the most widely used tranquillizers, is metabolized to chlorpromazine sulphoxide and this is isographic with DMPE (Fig. 2) in most of the usual chromatographic systems.

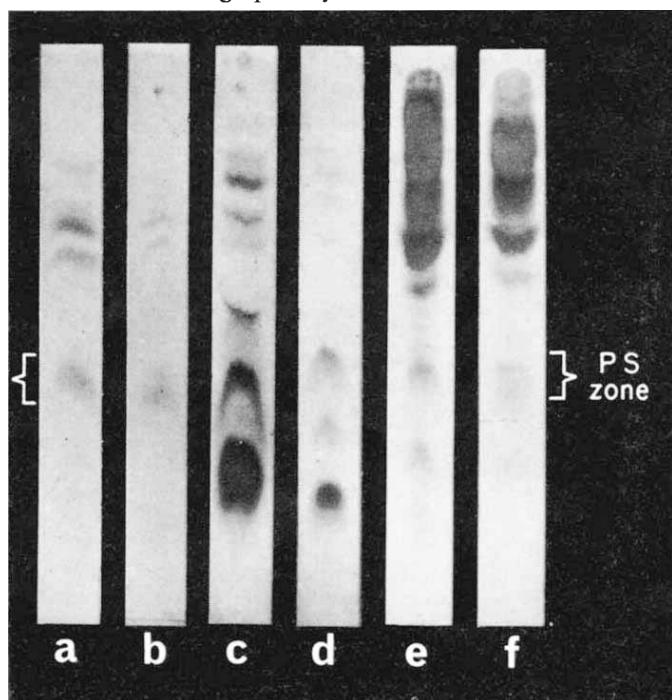


Fig. 2 Typical pink spot chromatograms produced after single dimension descending separation in the solvent system butanol : acetic acid : water (60 : 15 : 25, v/v). The chromogen in these examples is that produced after spraying with ninhydrin-pyridine reagent. A pink spot was produced in all cases in the appropriate zone after treatment with Ehrlich's reagent but photography at this second stage was not possible. Chromatograms *a* and *b* represent extracts ($\approx 200\text{ mg}$ and 25 mg creatinine respectively) from a patient with Parkinson's disease. Chromatograms *c* and *d* represent urine extracts ($\approx 200\text{ mg}$ and 25 mg of creatinine respectively) from a schizophrenic patient ingesting the tranquillizer chlorpromazine. Strips *e* and *f* are more concentrated extracts ($\approx 500\text{ mg}$ and 100 mg of creatinine respectively) from a non-drugged schizophrenic patient. DMPE ($5\text{ }\mu\text{g}$) was over-spotted on chromatograms *b*, *d* and *f*. Although a positive pink spot substance isographic with DMPE is clearly visible on strips *a*, *c* and *e*, it was not DMPE.

In an extensive survey of a normal and psychotic population Bourdillon *et al.*⁴⁹ showed that the presence of a pink spot seemed to be particularly associated with the "Schneider positive" non-paranoid⁹⁵ group of schizophrenics.

Clearly urinary DMPE does exist; it is excreted in small amounts, probably less than $10\text{ }\mu\text{g}/\text{day}$, and it is not unique to schizophrenia and does not seem to be increased in this disease.

The controversial and conflicting reports by reputable researchers that have centred on the presence and/or absence of DMPE and other pink spots have highlighted the great need for more positive identification of body fluid

and tissue factors in the future. At the last count Barbeau (personal communication) found seventeen pink spots in the close neighbourhood of DMPE in a urine extract obtained from Parkinson patients. Although it is inherently possible to obtain an unambiguous identification of an unknown substance by chromatography alone the time and effort involved would be considerable. In spite of the nature of substances of biological interest (very small quantities, considerable contamination and so on) it is now feasible to achieve absolute or near absolute identification by a more enlightened use of chemical laboratory technology (ultra-violet, infrared and mass spectroscopy, for example). In addition it is feasible^{50,51} and desirable to report the quantitative assessment of the excretion of the interesting metabolites.

Although methylation of catecholamines occurs *in vivo* it seems to occur predominantly at position 3 of the benzene nucleus. In attempts to locate the precursor of DMPE Friedhoff and van Winkle⁵² infused tritiated dopamine to schizophrenic patients; subsequent analysis of the urine showed that small quantities of labelled DMPE were excreted. Most of the label, however, occurred in the acid metabolite 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid) with just a little in DMPAA. Incubation of a liver biopsy sample with tritiated dopamine resulted in labelled homovanillic acid and DMPAA. Faurbye and Pind⁵³ in a similar experiment could not detect DMPE or DMPAA in the urine after infusion of tritiated dopamine to both normal and schizophrenic individuals. Kuehl *et al.*^{59,54} showed *in vitro* that methylation of dopamine in the 4 position could occur with about 25 per cent of the efficiency of 3-methylation. They could not, however, obtain any evidence for the formation of DMPE or DMPAA. A recent significant finding⁵⁵, at least for general metabolism, is that in the rat 4-demethylation is about fifteen times more rapid than 3-demethylation. It remains possible therefore that abnormal methylation occurs in certain susceptible regions of the brain and so upsets some processes subserving mentation. The subsequent demethylation would make it difficult if not impossible to observe the abnormal metabolite. The possibility of a highly localized cerebral malfunction is not limited, however, to abnormal methylation; almost any upset would escape notice on account of the tiny concentrations involved.

DMPE and Behaviour

Because of the potential role of DMPE in the aetiology of schizophrenia, many studies of the effects of this substance in animals are now under way. DMPE and other analogues of mescaline can produce catatonia^{56–58} and there is a correlation between the impairment of rope climbing ability in trained rats and the amount of DMPE in the brain following intraperitoneal injection⁵⁹. Smythies and Sykes⁶⁰ and Bergen⁶¹ have shown that DMPE alters rat behaviour in a way which is unusual but similar to the effects of an abnormal protein fraction taken from schizophrenics. Proctor *et al.*^{62,63} have recently shown that when DMPE is incubated with blood plasma from unmedicated acute schizophrenics and then injected into mice an amphetamine-like excitatory lethal response follows. The response was not obtained with unmedicated non-schizophrenic plasma or physiological saline in the incubation mixture instead. Dear and Malcolm⁶⁴ have investigated the cortical potentials evoked by DMPE administered both topically and systemically after stimulation of the right forepaw of rats and its ability to block the inhibitory action of other biogenic amines. Bindler *et al.*¹¹¹ have shown that the evoked cortical response in cats is depressed by intravenous injection of DMPE. Takeo and Himwich⁶⁵ claim that after intracarotid injection of DMPE an EEG arousal occurs in the lower brain stem of rabbits. This they compare with the

effect of mescaline, but distinguish from adrenaline where the arousal is at the mid-brain level. Brown *et al.*⁶⁶ have shown that intravenous injection to conscious and anaesthetized dogs and cats changes blood pressure. Barbeau *et al.*⁶⁷ showed that chronic injections of DMPE during prolonged periods produce a persistent akinetic syndrome in rats, rabbits and monkeys. They also showed that in the rat brain there seems to be an effect on dopamine metabolism, the dopamine levels being highest at the time of maximal hypokinetic effect⁶⁸, and homovanillic acid excretion in the urine being significantly elevated in the 24 h following injection⁶⁹.

Interesting as these animal studies are they cannot yet be related to schizophrenia because it has been shown that the ingestion of DMPE in man^{70,71}, even in high doses, produces no psychological changes. And there is no significant difference between schizophrenics and other groups in the urinary excretion of DMPE and its metabolites. Furthermore there have been no observed differences in the metabolism of the various catecholamines^{39,53,54,72-78} and this in spite of considerable searching.

As a final comment on this particular topic, Shulgin *et al.*⁷⁹, on the basis of dose-response relationships of various psychotomimetic phenylethylamines, say that if the idea of an endogenous psychotogen is acceptable then two very good bets are 3,4-dimethoxyphenylethanolamine and 2-hydroxy-4,5-dimethoxyphenylethanolamine (Fig 1), this latter arising as a result of β -hydroxylation and methylation of 6-hydroxy dopamine⁸⁰.

Indoleamines

Indolic substances have been clearly implicated in some conditions (Hartnups disease, pellagra, phenylketonuria) which are often accompanied by mental disturbances. Many psychotomimetic substances possess an indole nucleus, and feeding tryptophan after dosage with monoamine oxidase inhibitors apparently leads to an exacerbation of psychotic symptoms^{15,17}.

Benassi *et al.*⁸¹ have shown that, although there is no apparent difference between normals and schizophrenics in the spontaneous urinary excretion of tryptophan metabolites (and this seems to be the consensus of earlier reports), the urinary pattern in schizophrenics changes considerably after heavy tryptophan loading (490 μ moles/kg). In their cases the kynurenine pathway metabolites are about 240 per cent higher than in normals; the 5-hydroxyindoleacetic acid excretion was not significantly affected in either group. Ingestion of various vitamins of the B complex did not normalize the urinary excretion pattern, although Price *et al.*⁸² using less concentrated tryptophan loads found that it did. Sprince *et al.*^{21,83} have also reported an increased excretion of some tryptophan metabolites (indole acetamide and 6-hydroxyskatole) in schizophrenia and an increase in urinary indoles following methionine ingestion after dosage with a monoamine oxidase inhibitor.

The potential importance of certain vitamins and co-factors in schizophrenia was highlighted recently by Pauling⁸⁴. He argued that some individuals, particularly those who are mentally ill, may suffer from an inadequate supply of essential nutritional factors, if not in their diet then cerebrally, as a result of their individuality or disease. This is an important possibility and one that is amenable, to some extent at any rate, to investigation. Current evidence is not substantial although it is suggestive (see, for example, refs. 137-141), and nutritional disorders sometimes are heralded by mental changes. It is also interesting that Huszák and Durkó⁸⁵ claimed that there is much more of an indole-pyridoxal complex—not yet positively identified—in schizophrenic than in normal urine.

While investigating the effects of reserpine on the urinary excretion of tryptamine in schizophrenics, Himwich's group⁸⁷

found that in any spontaneous analysis concentrations of tryptamine were normal but in longitudinal studies there were occasions when they were very much higher than normal. These occasions coincide with increased psychotic behaviour. In later studies^{88,89} this Galesburg group have shown striking correlations between concentrations of urinary creatinine and tryptamine excreted, and report that creatinine excretion is by no means as constant as many believe.

Toxic Psychotogens

The fact that so many psychotomimetic compounds are methylated indoles has led many investigators to search in this area for the elusive toxic psychotogen. One such substance claimed to possess hallucinogenic properties and produce abnormal behaviour in animals (refs. 90, 91 and personal communication from E. Fischer) is bufotenin (N,N-dimethyl serotonin) (Fig 3): whether this substance is really a hallucinogen is controversial⁹².

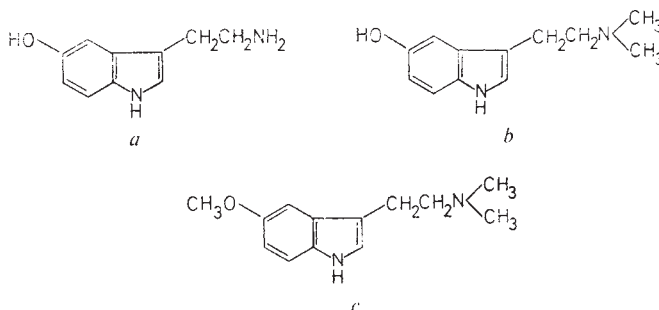


Fig. 3 Some indoleamines. *a*, 5-Hydroxytryptamine (serotonin); *b*, 5-hydroxy-N,N-dimethyltryptamine (bufotenin); *c*, 5-methoxy-N,N-dimethyltryptamine (O-methoxy bufotenin).

In 1955 Bumpus and Page⁹³ using physiological and chromatographic techniques obtained evidence suggesting the existence of serotonin, N-methyl serotonin and bufotenin in normal healthy urine extracts. Fischer *et al.*^{86,94} using non-specific unidimensional paper chromatographic methods claimed that a bufotenin-like substance was excreted uniquely by schizophrenic patients. Later publications from Fischer's group^{96,97} have reported different techniques based on the diazo coupling of the reactive substances which remain in a urine extract after oxidation of primary and secondary amines. Using this procedure Fischer's group claim that non-schizophrenics and chronic schizophrenics, medicated or unmedicated, excrete an average of 3.6 μ g/100 ml. of urine, acute unmedicated schizophrenics excrete significantly more (17.2 μ g/100 ml. of urine). This means that more than 50 μ g of bufotenin can be expected to be excreted each day in any urine. Although these concentrations seem reasonable when compared with the findings of Gross and Franzen^{98,99} for blood and urine, they are considerably in excess of the values quoted by other workers. Perry *et al.*¹⁰⁰ found by two-dimensional paper chromatography small amounts (<2.2 μ g/100 g of creatinine) in children during monoamine oxidase blockade. Rodnight¹⁰¹ using paper chromatographic and physiological techniques concluded that bufotenin, if present, would have to be in a concentration less than 5 μ g/24 h. Seigel¹⁰² found no evidence for bufotenin in either normal or schizophrenic urines; he also showed that the high results of Gross and Franzen^{98,99} could be produced by some other urinary fluorophore possessing a significant fluorescence at the peak fluorescing wavelength of the bufotenin fluorophore. We (in unpublished work) reached a similar conclusion using the colorimetric method described by Spatz⁹⁶. With this technique we found about 80 μ g of chromogen/1.5 g of creatinine in a normal urine specimen but nothing in the bufotenin zone on a thin-layer chromatogram. No evidence for urinary bufotenin could be

found by Runge *et al.*¹⁰³ and Nishimura and Gjessing³⁴. Brune *et al.*¹⁰⁴ reported that a substance isographic with bufotenin in several solvent systems occurred more frequently in acute unmedicated schizophrenics than in other individuals. This bufotenin-like substance was much increased when its oxidation was prevented by isocarboxiazid.

Increased interest in this problem has followed Axelrod's observation^{105,106} that an enzyme system in rabbit lung N-methylates tryptamine to N-methyltryptamine and N,N-dimethyltryptamine and 5-hydroxytryptamine to N-methyl serotonin and bufotenin.

Amines in Urine and Brain

Sanders and Bush¹⁰⁷ have shown in the rat that very little intravenously injected bufotenin reaches the brain. The excretion pattern in the urine was: unchanged bufotenin (6 per cent), bufotenin glucuronide conjugate (35 per cent) and 5-hydroxyindoleacetic acid (15 per cent). The O-methyl derivative of bufotenin reaches the brain quickly and is very rapidly metabolized. Gessner and Page¹⁰⁸ and Holmstedt¹⁰⁹ claim that O-methoxy bufotenin (5-methoxy-N,N-dimethyltryptamine, Fig 3) is a much more potent hallucinogen than bufotenin itself.

Himwich's group^{110,112}, expanding earlier studies of the excretion of methylated tryptamines, claim that a worsening of the mental and behavioural symptoms during cysteine loading and monoamine oxidase blockade in schizophrenic patients started about 2 weeks after the concentrations of bufotenin in the urine were seen to increase. These concentrations remained increased during periods of symptomatic exacerbations. More recently this group¹¹³ have examined six chronic male schizophrenics and four non-schizophrenics (mental defectives) by two-dimensional thin-layer techniques and claim that conjugated bufotenin was detectable in the urine of all ten subjects in a concentration estimated to be below 1 µg/24 h. The six schizophrenics and one defective also excreted free bufotenin. There seemed to be a relationship between the amount of free bufotenin and exacerbation of psychotic behaviour. This study, however, was conducted on a small number of cases and an absolutely positive identification of the urinary factors is still lacking. As McGeer's group³⁶ have shown, at very low concentrations, artefacts and other urinary constituents can easily be confused with the metabolite of interest. Faurbye and Pind¹¹⁴, after concentrating urinary amine extracts by ion-exchange chromatography and sublimation, found that a spot isographic with bufotenin after two-dimensional separation on thin-layer plates was present in both schizophrenic and normal individuals without any significant differences being apparent. These authors, however, investigated the total amount of bufotenin in the urine samples without differentiation between the free and conjugated forms.

In the context of phenylethylamines and indoleamines, it is important to mention the work of the group in Roskilde, Denmark, who have concentrated on the neurochemical and behavioural changes accompanying amphetamine injection in animals (see recent review by Faurbye¹¹⁵). Of all the drug-induced schizophrenic-like reactions, the amphetamine psychosis is apparently the closest¹¹⁶ and in fact it is difficult to distinguish chronic amphetamine intoxication from "genuine" schizophrenia. The effect of amphetamine in rats is to produce a stereotyped behaviour pattern¹¹⁷ which is independent of general motor activity; anti-psychotic drugs and some metabolic inhibitors inhibit the effect of the amphetamine¹¹⁷⁻¹²¹. This characteristic stereotyped behaviour is also produced by certain amines^{118,122-125} (β-phenylethylamine, tryptamine, dopamine) and amine precursors (5-hydroxytryptophan and dopa). Direct injection of dopamine into the corpus striatum causes the stereotypies even when overall dopamine synthesis is blocked by α-methyl-p-tyrosine.

Destruction of the corpus striatum¹¹⁹ prevents the production of the stereotypies. Faurbye¹¹⁵ extrapolates these findings to schizophrenia and suggests that the disease is caused by an increase in one or more naturally occurring amines.

The distribution of amines in the brain is not uniform and they undoubtedly fulfil important roles. As long ago as 1962 Sourkes¹²⁶ suggested that in the aetiology of mental illness possible amine imbalances were worthy of study. This idea certainly has proved valid in diseases affecting the basal ganglia: in Parkinson's disease at least the neurochemical findings have indicated a therapy which in some cases produces dramatic improvements¹²⁷. The work of the Galesburg group seems to implicate some indoleamines and the Roskilde group have demonstrated the importance of amines in some aspects of animal behaviour. All these findings suggest a continuous or transient dysfunction in phenylalanine and/or tryptophan metabolism. It seems sensible therefore to pursue studies of amine imbalance and the metabolism of the essential aromatic amino-acids in longitudinal studies of psychotics with carefully selected patients and adequate controls. Such a research approach is neither cheap nor easy but we know that a biochemical abnormality may lead to very complex metabolic changes which vary from case to case even in such a relatively clear cut condition as phenylketonuria¹²⁸. Furthermore, such an approach would make it unnecessary to chase elusive and hypothetical psychotogens.

Other Molecules

Greiner¹³⁰⁻¹³² has observed in some schizophrenic patients a skin discoloration and an associated eye pathology. He has postulated that in schizophrenia melanogenesis is increased and that the process is further augmented by chronic ingestion of phenothiazines; penicillamine therapy and a low copper diet are reported to lead to remarkable clinical improvement¹³².

Huszák and Durkó¹³³ from fluorimetric observations and paper chromatography have implicated disturbed porphyrin metabolism. Price *et al.*⁸² also reported disturbances in porphyrin metabolism in their schizophrenic patients. In this connexion it is interesting that Irvine *et al.* (personal communication) have identified the so-called mauve factor^{129,134,170} as a trialkylpyrrole. Although this mauve factor is excreted by some non-psychotics it does seem to be significantly related to psychoses^{135,136}; it has also been reported in the urine of some cancer patients¹³⁵.

Evidence suggesting an abnormality in schizophrenic plasma has a long and controversial history (see reviews in refs. 7, 8 and 142). Frohman *et al.*¹⁴³⁻¹⁴⁷ have claimed that incubation of schizophrenic plasma with chicken erythrocytes upsets carbohydrate metabolism: in particular it increases the lactate to pyruvate ratio. Attempts to repeat this work in several different laboratories were unsuccessful although other abnormalities were mentioned¹⁴⁸⁻¹⁵¹. Further data implicating abnormal carbohydrate metabolism have been reported by Walaas *et al.*^{152,153}: schizophrenic serum causes an inhibition in the utilization of glucose by the isolated rat diaphragm whereas normal serum stimulates utilization.

There are many conflicting reports of changes in albumin and globulin patterns^{154-156,158,166,167,175}, macroglobulins^{157,166,174}, haptoglobulins^{159,173}, plasminogen¹⁶⁰, fibrinogen¹⁶¹, lymphocytes¹⁶²⁻¹⁶⁴, and leucocytes¹⁶⁵ in schizophrenic blood. Numerous studies have illustrated that the injection of whole plasma or plasma fractions causes changes in the behaviour of trained animals^{168,169} although, as usual in this field, others have failed to confirm this^{171,172}.

The most extensive work in this whole area is that emanating from the group in Tulane University, New Orleans. They have isolated a fraction, called taraxein (a protein with perhaps an attached small molecule) claimed

to produce changes in the ability of rats to climb ropes, produce abnormal EEG activity from subcortical implanted electrodes in monkeys and produce schizophrenic-like behaviour in human volunteers¹⁷⁶⁻¹⁸⁰; these claims have to some extent been substantiated in other laboratories¹⁸¹⁻¹⁸⁶. The Tulane group has recently postulated that schizophrenia is an immunological disease in which taraxein (antibody) reacts with certain antigenic foci in the brain¹⁸⁷⁻¹⁹⁰. This is an attractive idea but there is currently very little supporting evidence.

A critical survey of any single experiment in this field of toxic factors in schizophrenic blood leaves considerable doubts as to the significance of the findings and their relevance to schizophrenia. If all the diverse experiments are considered, however, even including the lack of reproducibility between some of them, the feeling remains that there is probably an abnormality in the plasma taken from some schizophrenic patients. Lehman¹⁹¹, with particular reference to the taraxein literature, suggests that it would be highly desirable to have a system other than the central nervous system of primates for testing these abnormal fractions and that such work should now be undertaken in other centres.

I thank the Psychiatric Services Branch of the Province of Saskatchewan for continuing support and Dr G. L. Marjerrison for his help in the preparation of this review. This article was written in March 1969.

- ¹ Kallman, F. J., in *American Handbook of Psychiatry* (edit. by Arieti, S.), **1**, 175 (Basic Books, New York, 1959).
- ² Essen-Möller, E., *Acta Psychiat. Scand.*, **39**, 65 (1963).
- ³ Tienari, P., *Acta Psychiat. Scand.*, **39**, Suppl. 171 (1963).
- ⁴ Kringlen, E., *Acta Psychiat. Scand.*, **40**, Suppl. 180, 313 (1965).
- ⁵ Kringlen, E., *Psychiatry*, **29**, 172 (1966).
- ⁶ Heston, L. L., *Brit. J. Psychiat.*, **112**, 819 (1966).
- ⁷ Weil-Malherbe, H., *Adv. Enzymol.*, **29**, 479 (1967).
- ⁸ Rosenbaum, C., *J. Nerv. Ment. Dis.*, **146**, 103 (1968).
- ⁹ Huxley, J., Mayr, E., Osmond, H., and Hoffer, A., *Nature*, **204**, 220 (1964).
- ¹⁰ Kaplan, A. R., *Nature*, **210**, 870 (1966).
- ¹¹ Baldessarini, R. J., and Snyder, S. H., *Nature*, **206**, 1111 (1965).
- ¹² Moran, P. A. P., *Nature*, **206**, 1113 (1965).
- ¹³ Osmond, H., and Smythies, J., *J. Ment. Sci.*, **98**, 309 (1952).
- ¹⁴ Harley-Mason, J., *J. Ment. Sci.*, **98**, 313 (1952).
- ¹⁵ Pollin, W., Cardon, P. V., and Kety, S. S., *Science*, **133**, 104 (1961).
- ¹⁶ Park, L. C., Baldessarini, R. J., and Kety, S. S., *Arch. Gen. Psychiat.*, **12**, 346 (1965).
- ¹⁷ Alexander, F., Curtis, G. C., Sprince, H., and Crosley, A. P., *J. Nerv. Ment. Dis.*, **137**, 135 (1963).
- ¹⁸ Brune, G. C., and Himwich, H. E., *J. Nerv. Ment. Dis.*, **134**, 447 (1962).
- ¹⁹ Haydu, G. G., Dhrymiotis, A., Korenyi, C., and Goldschmidt, L., *Amer. J. Psychiat.*, **122**, 560 (1965).
- ²⁰ Brune, G. C., and Himwich, H. E., in *Recent Advances in Biological Psychiatry*, **5**, 144 (Plenum, 1963).
- ²¹ Sprince, H., in *Amines and Schizophrenia* (edit. by Himwich, H. E., Kety, S. S., and Smythies, J. R.) (Pergamon, 1967).
- ²² Hoffer, A., Osmond, H., Callbeck, M. J., and Kahan, I., *J. Clin. Exp. Psychopathol.*, **18**, 131 (1957).
- ²³ Denson, R., *Dis. Nerv. Syst.*, **23**, 1 (1962).
- ²⁴ Greenberg, D. M., *Adv. Enzymol.*, **25**, 395 (1963).
- ²⁵ Friedhoff, A. J., and van Winkle, E., *Nature*, **194**, 897 (1962).
- ²⁶ Boulton, A. A., and Felton, C., *Nature*, **211**, 1404 (1966).
- ²⁷ Takesada, M., Kakimoto, Y., Sano, I., and Kaneko, Z., *Nature*, **199**, 203 (1963).
- ²⁸ Takesada, M., Miyamoto, E., Kakimoto, Y., Sano, I., and Kaneko, Z., *Nature*, **207**, 1199 (1965).
- ²⁹ Von Studnitz, W., and Nyman, G. E., *Acta Psychiat. Scand.*, **41**, 117 (1965).
- ³⁰ Faurbye, A., and Pind, K., *Acta Psychiat. Scand.*, **40**, 240 (1964).
- ³¹ Pind, K., and Faurbye, A., *Acta Psychiat. Scand.*, **42**, 246 (1966).
- ³² Williams, C. H., Gibson, J. G., and McCormick, W. O., *Nature*, **211**, 1195 (1966).
- ³³ Perry, T. L., Hansen, S., and MacIntyre, L., *Nature*, **202**, 519 (1964).
- ³⁴ Nishimura, T., and Gjessing, L. R., *Nature*, **206**, 963 (1965).
- ³⁵ Sen, N. P., and McGeer, P. L., *Biochem. Biophys. Res. Commun.*, **14**, 227 (1964).
- ³⁶ Jones, R. L., Bory, P., Brown, W. T., and McGeer, P. L., *Canad. J. Biochem.*, **47**, 185 (1969).
- ³⁷ Perry, T. L., Hansen, S., MacDougall, L., and Schwarz, C. J., *Nature*, **212**, 146 (1966).
- ³⁸ Perry, T. L., Hansen, S., and MacDougall, L., *Nature*, **214**, 484 (1967).
- ³⁹ Kuehl, F. A., Hichens, M., Ormond, R. E., Meisinger, M. A. P., Gale, P. A., Cirillo, V. J., and Brink, N. G., *Nature*, **203**, 154 (1964).
- ⁴⁰ Kuehl, F. A., Ormond, R. E., and Vandenheuvel, W. J. A., *Nature*, **211**, 606 (1966).
- ⁴¹ Bell, C. E., and Somerville, A. R., *Nature*, **211**, 1405 (1966).
- ⁴² Boulton, A. A., and Felton, C., *Lancet*, **ii**, 964 (1966).
- ⁴³ Barbeau, A., De Groot, J. A., Joly, J. G., Raymond-Tremblay, D., and Donaldson, J., *Rev. Canad. Biol.*, **22**, 469 (1963).
- ⁴⁴ Boulton, A. A., Pollitt, R. J., and Majer, J. R., *Nature*, **215**, 132 (1967).
- ⁴⁵ Rinne, U. K., and Sonninen, V., *Nature*, **216**, 489 (1967).
- ⁴⁶ Creveling, C. R., and Daly, J. W., *Nature*, **216**, 190 (1967).
- ⁴⁷ Closs, K., Wad, N., and Ose, E., *Nature*, **214**, 483 (1967).
- ⁴⁸ Steinberg, H. R., and Robinson, J., *Nature*, **217**, 1054 (1968).
- ⁴⁹ Bourdillon, R. E., Clark, C. A., Ridges, A. P., Sheppard, P. M., Harper, P., and Leslie, N. G., *Nature*, **208**, 453 (1965).
- ⁵⁰ Boulton, A. A., *Second Intern. Cong. Neurogenetics and Neurophthalmology*, Montreal (1967), *Excerpta Medica*, No. 154, 35.
- ⁵¹ Boulton, A. A., in *Methods of Biochemical Analysis* (edit. by Glick, D.), **16**, 327 (Interscience, 1969).
- ⁵² Friedhoff, A. J., and van Winkle, E., *Nature*, **199**, 1271 (1963).
- ⁵³ Faurbye, A., and Pind, K., *Nature*, **215**, 1387 (1967).
- ⁵⁴ Wagner, A. F., Cirillo, V. J., Meisinger, M. A. P., Ormond, R. E., Kuehl, F. A., and Brink, N. G., *Nature*, **211**, 604 (1966).
- ⁵⁵ Sargent, T. W., Israelstam, D. M., Shulgin, A. T., Landaw, S. A., and Finlay, N. N., *Biochem. Biophys. Res. Commun.*, **29**, 126 (1967).
- ⁵⁶ de Jong, H., *Proc. Roy. Acad. Sci., Amsterdam*, **34**, II, 576 (1931).
- ⁵⁷ Noteboom, L., *Proc. Roy. Acad. Sci., Amsterdam*, **37**, II, 562 (1934).
- ⁵⁸ Ernst, A. M., *Nature*, **193**, 178 (1962).
- ⁵⁹ Vogel, W. H., and Horwitt, M. K., *Psychopharmacologia*, **11**, 265 (1967).
- ⁶⁰ Smythies, J. R., and Sykes, E. A., *Psychopharmacologia*, **8**, 324 (1966).
- ⁶¹ Bergen, J. R., *Trans. NY Acad. Sci.*, **28**, 40 (1965).
- ⁶² Proctor, C. D., Cho, J. B., Potts, J. L., Ashley, L. G., Douglas, J. G., Amoroso, C. P., McGriff, J. E., and Eaton, H. E., *Arch. Intern. Pharmacodyn.*, **172**, 95 (1968).
- ⁶³ Proctor, C. D., Cho, J. B., Ashley, L. G., Potts, J. L., Eaton, H. E., McGriff, J. E., Douglas, J. G., and Amoroso, C. P., *Arch. Intern. Pharmacodyn.*, **172**, 106 (1968).
- ⁶⁴ Dear, E. M. A., and Malcolm, J. C., *Intern. J. Neuropharmacol.*, **6**, 529 (1967).
- ⁶⁵ Takeo, Y., and Himwich, H. E., *Science*, **150**, 1309 (1965).
- ⁶⁶ Brown, M. L., Lang, W. J., and Gershon, S., *Arch. Intern. Pharmacodyn.*, **158**, 439 (1965).
- ⁶⁷ Barbeau, A., Tetreault, L., Oliva, L., Morazain, L., and Cardin, L., *Nature*, **209**, 719 (1966).
- ⁶⁸ Barbeau, A., Singh, P., Gaudreau, P., and Joubert, M., *Rev. Canad. Biol.*, **24**, 229 (1965).
- ⁶⁹ Barbeau, A., Lescop, J., Duplessis, P., and Elie, R., *Experientia*, **23**, 1 (1967).
- ⁷⁰ Hollister, L. E., and Friedhoff, A. J., *Nature*, **210**, 1377 (1966).
- ⁷¹ Shulgin, A. T., Sargent, T., and Naranjo, C., *Nature*, **212**, 1606 (1966).
- ⁷² Axelrod, J., *Biochim. Biophys. Acta*, **85**, 247 (1964).
- ⁷³ LaBrosse, E. H., Mann, J. D., and Kety, S. S., *J. Psychiat. Res.*, **1**, 68 (1961).
- ⁷⁴ Resnick, O., and Elmadjian, F., *Amer. J. Physiol.*, **187**, 626 (1956).
- ⁷⁵ Resnick, O., and Freeman, H., *Arch. Gen. Psychiat.*, **6**, 388 (1962).
- ⁷⁶ Pollin, W., and Goldin, S., *J. Psychiat. Res.*, **1**, 50 (1961).
- ⁷⁷ Cardon, P. V., and Mueller, A. S., *J. Psychiat. Res.*, **2**, 11 (1964).
- ⁷⁸ Cardon, P. V., Sokoloff, L., Vates, T. S., and Kety, S. S., *J. Psychiat. Res.*, **1**, 37 (1961).
- ⁷⁹ Shulgin, A. T., Sargent, T., and Naranjo, C., *Nature*, **221**, 537 (1969).
- ⁸⁰ Senoh, S., Creveling, C. R., Udenfriend, S., and Witkop, B., *J. Amer. Chem. Soc.*, **81**, 6236 (1959).
- ⁸¹ Benassi, C. A., Benassi, P., Allegri, G., and Ballarin, D., *J. Neurochem.*, **7**, 264 (1961).
- ⁸² Price, J. M., Brown, R. R., and Peters, H. A., *Neurology*, **9**, 456 (1959).
- ⁸³ Sprince, H., *Ann. NY Acad. Sci.*, **96**, 399 (1962).
- ⁸⁴ Pauling, L., *Science*, **160**, 265 (1968).
- ⁸⁵ Huszak, I., and Durko, I., *Acta Biochimica Polonica*, **11**, 389 (1964).
- ⁸⁶ Fischer, E., Vazquez, F. A., Fernández, T. A., and Liskowski, L., *Lancet*, **i**, 890 (1961).
- ⁸⁷ Brune, G. G., and Himwich, H. E., *Arch. Gen. Psychiat.*, **6**, 324 (1962).
- ⁸⁸ Berlet, H. H., Pscheidt, G. R., Spaide, J. K., and Himwich, H. E., *Nature*, **203**, 1198 (1964).

- ⁸⁹ Pscheidt, G. R., Berlet, H. H., Spaide, J., and Himwich, H. E., *Clin. Chim. Acta*, **13**, 228 (1966).
- ⁹⁰ Maire, F. W., and Hixon, A. C., *Intern. J. Neuropsychiat.*, **2**, 135 (1966).
- ⁹¹ Gessner, P. K., Khairallah, P. A., McIsaac, W. M., and Page, I. H., *J. Pharm. Exp. Therap.*, **130**, 126 (1960).
- ⁹² Fischer, R., *Nature*, **220**, 411 (1968).
- ⁹³ Bumpus, F. M., and Page, I. H., *J. Biol. Chem.*, **212**, 111 (1955).
- ⁹⁴ Fischer, E., Fernandez-Lagravere, T. A., Vázquez, A. J., and DiStefano, A. O., *J. Nerv. Ment. Dis.*, **133**, 441 (1961).
- ⁹⁵ Schneider, K., *Fortschr. Neurol. Psych.*, **25**, 487 (1957).
- ⁹⁶ Spatz, H., *Pren. Méd. Argent.*, **54**, 2116 (1967).
- ⁹⁷ Fischer, E., and Spatz, H., *Arch. Psychiat. Z. Neurol.*, **211**, 241 (1968).
- ⁹⁸ Franzen, F., and Gross, H., *Nature*, **206**, 1052 (1965).
- ⁹⁹ Gross, H., and Franzen, F., *Biochem. Z.*, **340**, 403 (1964).
- ¹⁰⁰ Perry, T. L., Shaw, K. N. F., Walker, D., and Redlich, D., *Pediatrics*, **30**, 576 (1962).
- ¹⁰¹ Rodnight, R., *Biochem. J.*, **64**, 621 (1956).
- ¹⁰² Seigel, M., *J. Psychiat. Res.*, **3**, 205 (1965).
- ¹⁰³ Runge, T. M., Lara, F. Y., Nawasa-Thurman, M. J., Keyes, J. W., and Hoerster, S. H., *J. Nerv. Ment. Dis.*, **142**, 473 (1966).
- ¹⁰⁴ Brune, G. G., Hohl, H. H., and Himwich, H. E., *J. Neuropsychiat.*, **5**, 14 (1963).
- ¹⁰⁵ Axelrod, J., *Science*, **134**, 343 (1961).
- ¹⁰⁶ Axelrod, J., *J. Pharmacol. Exp. Therap.*, **138**, 28 (1962).
- ¹⁰⁷ Sanders, E., and Bush, M. T., *J. Pharmacol. Exp. Therap.*, **158**, 340 (1967).
- ¹⁰⁸ Gessner, P. K., and Page, I., *Amer. J. Physiol.*, **203**, 167 (1962).
- ¹⁰⁹ Holmstedt, B., in *Amines and Schizophrenia* (edit. by Himwich, H. E., Kety, S. S., and Smythies, J. R.), 151 (Pergamon, Oxford, 1967).
- ¹¹⁰ Spaide, J., Tanimukai, H., Ginther, R., Bueno, J., and Himwich, H. E., *Life Sci.*, **6**, 551 (1967).
- ¹¹¹ Bindler, E., Sanghvi, I., and Gershon, S., *Arch. Intern. Pharmacodyn.*, **176**, 1 (1968).
- ¹¹² Tanimukai, H., Ginther, R., Spaide, J., Bueno, J. R., and Himwich, H. E., *Life Sci.*, **6**, 1697 (1957).
- ¹¹³ Tanimukai, H., Ginther, R., Spaide, J., and Himwich, H. E., *Nature*, **216**, 490 (1967).
- ¹¹⁴ Faurbye, A., and Pind, K., *Nature*, **220**, 489 (1968).
- ¹¹⁵ Faurbye, A., *Comp. Psychiat.*, **9**, 155 (1968).
- ¹¹⁶ Kety, S. S., in *Amines and Schizophrenia* (edit. by Himwich, H. E., Kety, S. S., and Smythies, J. R.), 272 (Pergamon, Oxford, 1967).
- ¹¹⁷ Randrup, A., Munkvad, I., and Udsen, P., *Acta Pharmacol. Toxicol.*, **20**, 145 (1963).
- ¹¹⁸ Munkvad, I., and Randrup, A., *Acta Psychiat. Scand.*, **42**, Suppl. 191, 178 (1966).
- ¹¹⁹ Randrup, A., and Munkvad, I., *Acta Psychiat. Scand.*, **42**, Suppl. 191, 193 (1966).
- ¹²⁰ Quinton, R., and Halliwell, G., *Nature*, **200**, 178 (1963).
- ¹²¹ Fog, R. L., Randrup, A., and Pakkenberg, H., *Psychopharmacologia*, **11**, 179 (1967).
- ¹²² Randrup, A., and Scheel-Kruger, J., *J. Pharm. Pharmacol.*, **18**, 752 (1966).
- ¹²³ Randrup, A., and Jonas, W., *J. Pharm. Pharmacol.*, **19**, 483 (1967).
- ¹²⁴ Randrup, A., and Munkvad, I., *Nature*, **211**, 540 (1966).
- ¹²⁵ Scheel-Kruger, J., and Randrup, A., *Life Sci.*, **6**, 1389 (1967).
- ¹²⁶ Sourkes, T. L., in *Neurochemistry*, second ed. (edit. by Elliott, K. A. C., Page, I. H., and Quastel, J. H.) (Thomas, Springfield, 1962).
- ¹²⁷ Cotzias, G. C., and Papavasiliou, P. S., in *Second Intern. Cong. Neurogenetics and Neuroophthalmology*, Montreal, 1967, *Excerpta Medica*, No. 154, 30 (1967).
- ¹²⁸ Fuller, R. N., and Shuman, J. B., *Nature*, **221**, 639 (1969).
- ¹²⁹ Sohler, A., Renz, R. H., Smith, S., and Kaufman, J., *Intern. J. Neuropsychiat.*, **3**, 327 (1967).
- ¹³⁰ Greiner, A. C., *Dis. Nerv. Syst.*, **29**, 14 (1968).
- ¹³¹ Greiner, A. C., and Nicolson, G. A., *Lancet*, **ii**, 1165 (1965).
- ¹³² Nicholson, G. A., Greiner, A. C., McFarlane, W. J. G., and Baker, R. A., *Lancet*, **i**, 344 (1966).
- ¹³³ Huszák, I., and Durkó, I., *Proc. Fourth World Cong. Psychiat.*, Madrid, 1966, *Excerpta Med.*, No. 150, 2931 (1966).
- ¹³⁴ Irvine, D. G., *J. Neuropsychiat.*, **2**, 292 (1961).
- ¹³⁵ O'Reilly, P. O., Ernest, M., and Hughes, G., *Brit. J. Psychiat.*, **111**, 741 (1965).
- ¹³⁶ O'Reilly, P. O., Hughes, G., Russel, S., and Ernest, M., *Dis. Nerv. Syst.*, **26**, 562 (1965).
- ¹³⁷ O'Brien, D., and Jensen, C. B., *Clin. Sci.*, **24**, 179 (1963).
- ¹³⁸ Hunter, R., and Mathews, D. M., *Lancet*, **ii**, 738 (1965).
- ¹³⁹ *Nature*, **221**, 410 (1969).
- ¹⁴⁰ Reynolds, E. H., *Brit. J. Psychiat.*, **113**, 911 (1967).
- ¹⁴¹ Reynolds, E. H., Chanarin, I., Milner, G., and Mathews, D. M., *Epilepsia*, **7**, 261 (1966).
- ¹⁴² Kety, S. S., *Science*, **129**, 1528 (1959).
- ¹⁴³ Beckett, P. G. A., Senf, R., Frohman, C. E., Tourney, G., and Gottlieb, J. S., *Amer. J. Psychiat.*, **118**, 959 (1962).
- ¹⁴⁴ Frohman, C. E., Czajkowski, N. P., Luby, E. D., Gottlieb, J. S., and Senf, R., *Arch. Gen. Psychiat.*, **2**, 263 (1960).
- ¹⁴⁵ Frohman, C., Luby, E. D., Tourney, G., Beckett, P. G. S., and Gottlieb, J. S., *Amer. J. Psychiat.*, **5**, 401 (1960).
- ¹⁴⁶ Frohman, C. E., Goodman, M., Beckett, P. G. S., Latham, L. K., Senf, R., and Gottlieb, J. S., *Ann. NY Acad. Sci.*, **96**, 438 (1962).
- ¹⁴⁷ Latham, K., Warner, K., Frohman, C., and Gottlieb, J., *Fed. Proc.*, **21**, 415 (1962).
- ¹⁴⁸ Buhler, D. R., and Ihler, G. S., *J. Lab. Clin. Med.*, **62**, 306 (1963).
- ¹⁴⁹ Mangoni, A., Balazs, A., and Coppen, A. J., *Brit. J. Psychiat.*, **109**, 231 (1963).
- ¹⁵⁰ Brown, F. C., *Arch. Gen. Psychiat.*, **10**, 409 (1964).
- ¹⁵¹ Ryan, J. W., Brown, J. D., and Durell, J., *Science*, **151**, 1408 (1966).
- ¹⁵² Walaas, O., Lingjaerde, O., Loken, E., and Hundevadt, E., *Scand. J. Clin. Lab. Invest.*, **6**, 245 (1954).
- ¹⁵³ Walaas, O., Walaas, E., Søvik, O., Alertsen, A. R., and Lindjaerde, O., *Conf. Neurol.*, **25**, 175 (1965).
- ¹⁵⁴ Fessel, W. J., *Arch. Gen. Psychiat.*, **6**, 132 (1962).
- ¹⁵⁵ Fessel, W. J., *Arch. Gen. Psychiat.*, **8**, 614 (1963).
- ¹⁵⁶ Gammack, D. B., and Hector, R. I., *Clin. Sci.*, **28**, 469 (1965).
- ¹⁵⁷ Fessel, W. J., Kurland, H. D., and Cutler, R. P., *Arch. Intern. Med.*, **113**, 669 (1964).
- ¹⁵⁸ Fessel, W. J., *Arch. Gen. Psychiat.*, **7**, 427 (1962).
- ¹⁵⁹ Seal, U. S., and Eist, H., *Clin. Chem.*, **12**, 709 (1966).
- ¹⁶⁰ Seal, U. S., and Swaim, W. R., *Clin. Chem.*, **14**, 368 (1968).
- ¹⁶¹ Seal, U. S., Swaim, W. R., and Eist, H., *Clin. Chem.*, **13**, 160 (1967).
- ¹⁶² Fessel, W. J., Hirata-Hibi, M., and Shapiro, I. M., *J. Psychiat. Res.*, **3**, 275 (1965).
- ¹⁶³ Vanderkamp, H., and Daly, R., *J. Neuropsychiat.*, **4**, 4 (1968).
- ¹⁶⁴ Hollister, L. L., and Kosek, J. C., *Intern. J. Neuropsychiat.*, **1**, 559 (1965).
- ¹⁶⁵ Fessel, W. J., and Hirata-Hibi, M., *Arch. Gen. Psychiat.*, **9**, 601 (1963).
- ¹⁶⁶ Fessel, W. J., and Grunbaum, B. W., *Ann. Intern. Med.*, **54**, 1134 (1961).
- ¹⁶⁷ Solomon, G. F., Moos, R. H., Fessel, W. J., and Morgan, E. E., *Intern. J. Neuropsychiat.*, **2**, 20 (1966).
- ¹⁶⁸ Bergen, J. R., Pennell, R. B., Freeman, H., and Hoagland, H., *Amer. Med. Assoc. Arch. Neurol.*, **2**, 146 (1960).
- ¹⁶⁹ Bishop, M. P., *Dis. Nerv. Syst.*, **21**, 1 (1960).
- ¹⁷⁰ Hoffer, A., and Osmond, H., *Acta Psychiat. Scand.*, **39**, 335 (1963).
- ¹⁷¹ Throne, M. L., Gowdey, C. W., and Lovegrove, T. D., *J. Nerv. Ment. Dis.*, **142**, 248 (1966).
- ¹⁷² Stewart, C. N., and Irvine, D. G., *Dis. Nerv. Syst.*, **23**, 456 (1962).
- ¹⁷³ Lovegrove, T. D., and Nicholls, D. M., *J. Nerv. Ment. Dis.*, **141**, 195 (1965).
- ¹⁷⁴ Sapira, J. D., *Arch. Gen. Psychiat.*, **10**, 196 (1964).
- ¹⁷⁵ Jensen, K., Clausen, J., and Osterman, E., *Acta Psychiat. Scand.*, **40**, 280 (1964).
- ¹⁷⁶ Heath, R. G., Martens, S., Leach, B. E., Cohen, M., and Fengley, C. A., *Amer. J. Psychiat.*, **114**, 917 (1958).
- ¹⁷⁷ Heath, R. G., Martens, S., Cohen, M., and Angel, C., *Amer. J. Psychiat.*, **114**, 14 (1957).
- ¹⁷⁸ Heath, R. G., *Intern. Rev. Neurobiol.*, **1**, 300 (1959).
- ¹⁷⁹ Heath, R. G., and Leach, B. E., *Ann. NY Acad. Sci.*, **96**, 425 (1962).
- ¹⁸⁰ Leach, B. E., Byers, L. W., and Heath, R. G., *Serological Fractions in Schizophrenia* (edit. by Heath, R. G.), (Harper and Row, New York, 1963).
- ¹⁸¹ Melander, B., and Martens, S., *Dis. Nerv. Syst.*, **19**, 478 (1958).
- ¹⁸² Nelson, J. W., Daniels, E. G., Mann, K. M., and Hinman, J. W., *Serological Fractions in Schizophrenia* (edit. by Heath, R. G.), 43 (Harper and Row, New York, 1963).
- ¹⁸³ Winter, C. A., Flataker, L., Boyer, W. P., Smith, E. V. C., and Sanders, B. E., in *Chemical Pathology of Nervous System* (edit. by Folch-Pi, J.), 64 (Pergamon, New York, 1961).
- ¹⁸⁴ Bergen, J. R., Pennell, R. B., Saravis, C. A., and Hoagland, H., in *Serological Fractions in Schizophrenia* (edit. by Heath, R. G.), 67 (Harper and Row, New York, 1963).
- ¹⁸⁵ Bergen, J. R., Gray, F. W., Pennell, R. B., Freeman, H., and Hoagland, N., *Arch. Gen. Psychiat.*, **12**, 80 (1965).
- ¹⁸⁶ Mekler, L. B., Laptera, N. N., Lozovski, D. V., and Balezina, T. I., *Dokl. Akad. Nauk. SSSR*, **130**, 148 (1960).
- ¹⁸⁷ Heath, R. G., and Krupp, I. M., *Arch. Gen. Psychiat.*, **16**, 3 (1967).
- ¹⁸⁸ Heath, R. G., Krupp, I. M., Byers, L. W., and Liljekvist, J. I., *Arch. Gen. Psychiat.*, **16**, 10 (1967).
- ¹⁸⁹ Heath, R. G., Krupp, I. M., Byers, L. W., and Liljekvist, J. I., *Arch. Gen. Psychiat.*, **16**, 24 (1967).
- ¹⁹⁰ Heath, R. G., and Krupp, I. M., *Amer. J. Psychiat.*, **124**, 1019 (1968).
- ¹⁹¹ Lehman, H. E., *Amer. J. Psychiat.*, **124**, 1024 (1968).

Carbon Chemistry of the Lunar Surface

P. H. CADOGAN, G. EGLINTON, J. R. MAXWELL & C. T. PILLINGER

Organic Geochemistry Unit, School of Chemistry, University of Bristol

Indigenous CH₄ and the chemical reaction product CD₄ released by deuterated acid etch of Apollo 11 and 12 lunar samples have been examined by gas chromatography. Possible origins for the CH₄ include solar wind synthesis and a small primordial contribution. The CD₄ probably arises from carbide or "carbide-like" materials contributed by meteorite impact and solar wind implantation.

We have suggested¹⁻³ that carbon and hydrogen implanted by the solar wind into the surface of lunar dust particles could give rise to methane and other hydrocarbons of low molecular weight. Indeed, "saturation" of the outer 1,000 Å of the lunar fines by nuclides in the solar wind⁴ might contribute a large part of the carbon content^{5,6} of the fines, and give rise to an extensive and varied range of chemical species.

Methane is evolved by crushing or acid treatment of the lunar fines^{1,2,7}. A deuterium labelling technique² enabled us to show that this methane has two components—indigenous gas, as we had suggested¹, and a contribution arising from acid-hydrolysable carbon compounds, probably carbides⁷, as Chang *et al.* suggested. This was done by treating lunar fines with DCl in D₂O, and analysing the gases released (chiefly CH₄ and CD₄) by low resolution mass spectrometry. Chang *et al.*⁸ have confirmed these results.

We have devised an improved method for gas chromatographic separation of CH₄ and CD₄, C₂H₆ and C₂D₆, CO, CO₂ and several other species, and have used it to confirm and extend our results³. The sensitivity allows us to analyse the gases released from 50 mg samples of lunar fines, and the method

could be applied to other materials (meteorites, terrestrial igneous rocks, glasses, metals and so on) containing trapped and/or reactive moieties in a matrix which can be attacked or dissolved by a suitably labelled reagent.

Only the quantities of CH₄ and CD₄ released by DF etch are discussed here. The samples examined and the quantities of these gases measured are listed in Table 1.

Origin of Indigenous Methane

We are endeavouring to discover whether a proportion of the CH₄ in the lunar fines derives from solar wind implantation. Table 1 lists the relevant data; correlations are indicated between the CH₄ content and the following factors.

(i) Total carbon content^{5,6}: samples with a high total carbon abundance have a correspondingly high CH₄ content; this relationship also holds for size-different fines. Moore *et al.*⁵ have observed that the Apollo 11 fines are richer in total carbon content than the igneous rocks and that, for the fines, the total carbon abundance increased with decreasing particle size. Our mass spectrometric data obtained by DCl etch² of size differentiated Apollo 11 fines indicate that CH₄ also seems to be concentrated in the smallest fines. These observations suggest that a surface-correlated phenomenon may be the source of some of the carbon in the fines⁵ and, in particular, of a proportion of the indigenous hydrocarbons.

(ii) The ³⁶Ar content (R. O. Pepin, private communication and ref. 13): examination of a number of sieved fractions from the Apollo 11 fines has shown that the rare gases are surface correlated¹⁸; shallow etching experiments confirm that they are concentrated within the outer 0.2 µm of the grains¹⁸. These two observations suggest that a large part of the ³⁶Ar observed in the fines is a result of solar wind implantation.

(iii) The fraction⁹ of mineral grains (in the 400 mesh residue) with an amorphous coating: the coating is highly disordered compared with the crystalline interior and has been tentatively attributed^{9,19} to solar wind implantation. But these grains could also represent cosmic dust irradiated in space and accreted by the Moon²⁰. The grains have a high latent track density

Table 1 Quantities of CH₄ and CD₄ released from Apollo 11 and 12 Lunar Fines by DF Etch* with Total Carbon Content and other Measurements relating to Surface Exposure

Sample No.	CH ₄ (µg/g)	CD ₄ (µg/g)	Total carbon ⁶ (µg g ⁻¹)	³⁶ Ar (10 ⁻⁸ cm ³ g ⁻¹)†	Fraction of grains with amorphous coating in 400 mesh residue ⁹	Fraction of grains with track density ¹⁰ ≥ 10 ⁸ cm ⁻²	Degree of reworking of regolith ^{11,12}	δ ¹³ C per mil relative to PDB standard ‡
Apollo 11								
bulk fines	4.8–5.2 ^a	18–22 ^a	140–226 ^b	38,000 ^c	67% ^d	> 0.9 ^e	high	+20.2 ^f
12042	2.1	15	130, 140	28,000	n.d.	0.92	n.d.	+11.2 ^g
12001	2.2	12	130	n.d.	n.d.	0.68	high	n.d.
12023	2.1	8	135, 166	n.d.	n.d.	n.d.	n.d.	+12.4 ^h
12037	1.2	3	82	n.d.	n.d.	0.52	intermediate	n.d.
12032	0.28	1.7	25, 60	4,200	15%	0.17	low	–1.6 ^h
12033	0.11	0.8	23, 50	2,800	n.d.	0.03	low	–3.6 ^g

* Gases released by etching¹⁻³ were fractionated at –196° C and the non-condensed fraction analysed at –78° C by gas chromatography (25' × 1/16" stainless steel, packed with 60–80 mesh Graphon, He flow rate 2 ml. min⁻¹).

† Highest value measured; this is the measurement least likely to be affected by terrestrial contamination¹⁵.

‡ Personal communication from R. O. Pepin.

n.d., Not determined.

a, 10,086D; b, 10,086A, 10,086B, ref. 5; c, 10,084, ref. 13; d, 10,084; e, 10,084, ref. 14; f, 10,086A, ref. 15; g, ref. 16; h, ref. 17.

(>10¹⁰ tracks cm⁻²), attributable to ions with a very short range, possibly solar suprathermal ions.

(iv) The solar flare cosmic ray track density: it has been suggested¹⁰ that grains with a high track density (>5×10⁸ tracks cm⁻²) have been exposed "unshielded" on the lunar surface, that is, "unshielded" by even one grain. On a statistical basis, it is likely that a sample which has a high fraction of grains with a solar flare cosmic ray track density >10⁸ tracks cm⁻² contains a large fraction of particles which have been exposed to the solar wind. The highest abundance of CH₄ is observed in samples having the highest fractions of particles with track densities >10⁸ tracks cm⁻².

(v) The degree of reworking of the regolith^{11,12}, that is, the slow "gardening" and mixing of the surface layer, occurring between sudden "blanket" depositions of layers of ejecta: the proportion of glassy spherules, microbreccias and glassy aggregates, which seem to be of impact origin, may be considered as a measure of the exposure time of a sample to an immediate surface environment. Comminution processes resulting from micrometeorite impact and other cosmic processes abrade the surface of grains: therefore, the average size distribution decreases with increased time on the surface. The results of Anders *et al.*²¹ for the siderophile elements Au and Ir and the volatile elements Zn, Cd, Bi and Se also suggest different exposure times. The contribution of carbonaceous chondrite type I material follows the same trend (10084>12037>12032>12033) as the degree of reworking of the regolith estimated from morphology. A high CH₄ abundance is observed with samples which show the greatest degree of reworking.

Etching of Surface of Lunar Grains

A solar wind origin for CH₄ would require that the gas be located in individual grains at a depth of <1000 Å. The rate of etch of meteoritic silicate grains in NaOH is slow at high temperatures²² and must be even slower at ambient. We therefore selected NaOD (40% in D₂O) as a reagent for shallow etching. Gas evolution is vigorous when the fines are treated with DF or DCl and the residue is an off-white flocculent material; in contrast, there was no visible evolution of gas when two samples of Apollo 11 fines were treated with NaOD, and the samples appeared to be unaltered. But more than 60% of the CH₄ which could be released by deuterated acids was released by the NaOD, indicating that a significant proportion of the CH₄ may be located at the surface of the fines. We are unable at present to measure accurately the depth of etch, although this depends both on the mineralogy and on the track densities. Further studies with selective etching agents are in progress.

Solar wind activity may not, however, be the only source of indigenous CH₄. Analysis of the gases released by a DF etch of crushed samples of interior chips of the basaltic rock 12022 (total carbon⁵ 50 µg g⁻¹) indicated the presence of CH₄ (about 1 µg g⁻¹) and C₂H₆ (about 0.1 µg g⁻¹), presumably of primordial origin and trapped during crystallization. Thus there is possibly a small primordial component of CH₄ in the fines although losses of this component during the formation of the fines are difficult to assess. More data from a variety of crystalline rocks are necessary.

It is unlikely that meteoritic CH₄ makes a significant contribution to the indigenous CH₄. The contribution to the lunar fines of carbonaceous chondrite type I material is of the order of 1–2%²¹ and the concentration of CH₄ in carbonaceous meteorites is low²³. Moreover, the gas loss caused by impact at undiminished cosmic velocities might be expected to be severe. Some meteoritic carbon might contribute indirectly to the CH₄ content through hydrogenation by solar wind hydrogen^{1,2}.

One further possibility is that pristine lunar fines possess highly reactive surface coatings which react with initially adsorbed terrestrial water¹⁶, and generate the CH₄ and other

light hydrocarbons observed. Completely unambiguous proof for or against this origin is unavailable. The hypothesis is not supported, however, by the lack of further reaction of fines with liquid water at 20° C (ref. 2).

Origin of Lunar Carbides

All the samples of lunar fines examined release CD₄ when treated with deuterated acids and are, therefore, presumed to contain carbides or "carbide-like" materials. In contrast, the single crystalline rock (12022) did not release CD₄, indicating an extralunar origin for these materials in the fines. Mineralogical examinations of the Apollo 11 fines have indicated the presence of the carbide, cohenite [(Fe, Ni)₃C]²⁴. These microscopic grains are almost certainly of meteoritic origin. From Anders's data²¹ concerning type I carbonaceous chondrite material, different samples of fines might also be expected to contain different contributions from the debris of cohenite-containing meteorites (coarse octahedrites²⁵). It is impossible to estimate by microscopic methods the meteoritic contribution to the carbide in the fines. But the results of two etching experiments lead us to believe that it is not large. First, we observed that DF etch of a sample of genuine meteoritic cohenite yields only 1–2% of the total carbon content as CD₄. Therefore, assuming a similar yield, the fines would require total carbon contents an order of magnitude in excess of those observed to account for the CD₄ evolved by acid treatment. A significant proportion of the carbide in the fines is possibly present in an extremely finely divided form, and not in discrete mineral grains. Second, δ¹³C isotopic ratio measurements by Chang *et al.*⁸ of the total hydrocarbons released by HCl etch of 12023 fines suggest that, on the whole, lunar carbide is different from known meteoritic carbide. These authors suggest that their observation could be explained by a "hydrogen stripping" process^{26,27} leading to a ¹³C enrichment, or by a contribution from another source.

An alternative extralunar origin for the carbide would be from the solar wind, and we propose that some of the carbide, like the CH₄, derives from this source. Thus, the trends outlined between CH₄ content and total carbon content^{5,6}, particle size², ³⁶Ar content¹³, fractions of grains with an amorphous coating (in the 400 mesh residue)⁹, the fraction of grains with a track density¹⁰ ≥ 10⁸ cm⁻² and the degree of reworking of the regolith^{11,12} also seem to be applicable to the CD₄ released (Table 1). The trends observed for CD₄ seem to be valid, although the CD₄/CH₄ ratios vary between samples. Carbon atoms implanted by the solar wind into available^{24,28} metal grains and inclusions and mineral grains in the fines could generate submicroscopic entities with the stoichiometry of carbides (see Chang *et al.*⁸). Confirmation of a solar origin for this "carbide-like" material could be provided by DF or DCl etch of metal or mineral grains selected from the fines and known^{28,29} to originate from the igneous rocks. At present, our methods are not sufficiently sensitive to perform this measurement.

To sum up, our data indicate the following: (i) a contribution to the indigenous hydrocarbons in the fines, which is a result of solar wind implantation; (ii) the presence of CH₄ of magmatic origin in the crystalline rock 12022 and, therefore, a possible small primordial contribution to the CH₄ in the fines; (iii) a dual extralunar origin (meteoritic and solar wind) for the carbide or "carbide-like" materials in the fines. The carbon chemistry of the lunar samples seems to be very dependent on the processes operating at the lunar surface³⁰. For example, there seems to be a relationship between the CH₄ content of the lunar fines and the δ¹³C value obtained for the total carbon content^{15–17} (Table 1). This observation supports the proposition that "hydrogen stripping" by solar wind hydrogen enriches the fines with respect to the heavier isotopes of carbon (¹³C, refs. 26 and 27), sulphur (³⁴S, refs. 26 and 27), oxygen (¹⁸O, ref. 16) and silicon (³⁰Si, ref. 16). The mechanism suggested involves preferential removal of the lighter isotopes

by conversion to volatile compounds (for example, $^{12}\text{C} \rightarrow ^{12}\text{CH}_4$) which are subsequently lost from the surface of the Moon. Further studies of a variety of samples are necessary to provide a detailed understanding of the processes occurring at the lunar surface, which must be widely applicable to this and other planetary systems.

We thank the SRC, NERC and NASA for financial assistance, and SRC for a fellowship (to C. T. P.) and PRF-ACS for a research studentship (to P. H. C.). We also thank Dr M. Maurette of the Centre de Spectrometrie de Mass du CNRS for criticisms of our manuscript and Dr R. O. Pepin of the University of Minnesota for unpublished results.

Received April 14, 1971.

- ¹ Abell, P. I., Draffan, G. H., Eglinton, G., Hayes, J. M., Maxwell, J. R., and Pillinger, C. T., *Proc. Apollo 11 Lunar Sci. Conf.* (edit. by Levinson, A. A.), 2, 1757 (Pergamon, London, 1970).
- ² Abell, P. I., Eglinton, G., Maxwell, J. R., Pillinger, C. T., and Hayes, J. M., *Nature*, **226**, 251 (1970).
- ³ Abell, P. I., Cadogan, P. H., Eglinton, G., Maxwell, J. R., and Pillinger, C. T., *Proc. Apollo 12 Lunar Sci. Conf.* (in the press).
- ⁴ Unsöld, A. O. J., *Science*, **169**, 1915 (1969).
- ⁵ Moore, C. B., Gibson, E. K., Larimer, J. W., Lewis, C. F., and Nichiporuk, W., *Proc. Apollo 11 Lunar Sci. Conf.* (edit. by Levinson, A. A.), 2, 1375 (Pergamon, London, 1970).
- ⁶ Moore, C. B., Lewis, C. F., Delles, F. M., Golley, R. C., and Gibson, E. K., *Proc. Apollo 12 Lunar Sci. Conf.* (in the press).
- ⁷ Chang, S., Smith, J. W., Kaplan, I., Lawless, J., Kvenvolden, K. A., and Ponnampuruma, C., *Proc. Apollo 11 Lunar Sci. Conf.* (edit. by Levinson, A. A.), 1857 (Pergamon, London, 1970).
- ⁸ Chang, S., Kvenvolden, K. A., Lawless, J., Ponnampuruma, C., and Kaplan, I. R., *Science*, **171**, 474 (1971).
- ⁹ Borg, J., Durrieu, L., Jouret, C., and Maurette, M., *Proc. Apollo 12 Lunar Sci. Conf.* (in the press).
- ¹⁰ Bhandari, N., Bhat, S., Lal, D., Rajagopalan, G., Tamhane, A. S., and Venkatavaradan, V. S., *Proc. Apollo 12 Lunar Sci. Conf.* (in the press).
- ¹¹ Quaide, W., Oberbeck, V., Bundi, T., and Polkowski, G., *Proc. Apollo 12 Lunar Sci. Conf.* (in the press).
- ¹² McKay, D., Morrison, D., Lindsay, J., and Ladle, G., *Proc. Apollo 12 Lunar Sci. Conf.* (in the press).
- ¹³ Pepin, R. O., Nyquist, L. E., Phinney, D., and Black, D. C., *Proc. Apollo 11 Lunar Sci. Conf.* (edit. by Levinson, A. A.), 2, 1435 (Pergamon, London, 1970).
- ¹⁴ Lal, D., MacDougall, D., Wilkening, L., and Arrhenius, G., *Proc. Apollo 11 Lunar Sci. Conf.* (edit. by Levinson, A. A.), 3, 2295 (Pergamon, London, 1970).
- ¹⁵ Kaplan, I. R., Smith, J. W., and Ruth, E., *Proc. Apollo 11 Lunar Sci. Conf.* (edit. by Levinson, A. A.), 2, 1317 (Pergamon, London, 1970).
- ¹⁶ Epstein, S., and Taylor, H. P., *Proc. Apollo 12 Lunar Sci. Conf.* (in the press).
- ¹⁷ Kaplan, I. R., and Petrowski, C., *Proc. Apollo 12 Lunar Sci. Conf.* (in the press).
- ¹⁸ Eberhardt, P., Geiss, J., Graff, H., Grögler, N., Krähenbühl, U., Schwaller, H., Schwarzmüller, J., and Settler, A., *Proc. Apollo 11 Lunar Sci. Conf.* (edit. by Levinson, A. A.), 2, 1037 (Pergamon, London, 1970).
- ¹⁹ Dran, J. C., Durrieu, L., Jouret, C., and Maurette, M., *Earth Planet. Sci. Lett.*, **9**, 391 (1970).
- ²⁰ Barber, D. J., Hutcheon, I., and Price, P. B., *Science*, **171**, 372 (1971).
- ²¹ Anders, E., Lane, J. C., Keays, R. R., Ganapathy, R., and Morgan, J. W., *Proc. Apollo 12 Lunar Sci. Conf.* (in the press).
- ²² Lal, D., Muralli, A. V., Rajan, R. S., Tamhane, A. S., Lorin, J. S., and Pellas, P., *Earth Planet. Sci. Lett.*, **5**, 111 (1968).
- ²³ Belsky, T., and Kaplan, I. R., *Geochim. Cosmochim. Acta*, **34**, 257 (1970).
- ²⁴ Fronzel, C., Klein, C., Ito, J., and Drake, J. E., *Proc. Apollo 11 Lunar Sci. Conf.* (edit. by Levinson, A. A.), 1, 445 (Pergamon, London, 1970).
- ²⁵ Brett, R., *Geochim. Cosmochim. Acta*, **31**, 143 (1967).
- ²⁶ Berger, R., *Nature*, **226**, 738 (1970).
- ²⁷ Kaplan, I. R., and Smith, J. W., *Science*, **167**, 541 (1970).
- ²⁸ Goldstein, J. I., and Yakowitz, H., *Proc. Apollo 12 Lunar Sci. Conf.* (in the press).
- ²⁹ Reid, A. M., Meyer, C., Harmon, R. S., and Brett, R., *Earth Planet. Sci. Lett.*, **9**, 1 (1970).
- ³⁰ Draffan, G. H., Eglinton, G., Hayes, J. M., Maxwell, J. R., and Pillinger, C. T., *Chem. in Britain*, **5**, 296 (1969).

Incorporation of High Molecular Weight Polynucleotides by Isolated Mitochondria

RONALD F. SWANSON*

Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland

Apparently intact mitochondria from ovaries of *Xenopus laevis* can incorporate high molecular weight polynucleotides which then serve as templates for polypeptide synthesis on mitochondrial ribosomes.

MITOCHONDRIA from a wide variety of organisms contain DNA¹, and there are two lines of evidence which indicate that this contains information for the synthesis of mitochondrial-specific structures. First, RNA from mitochondrial-specific ribosomes of *Neurospora crassa*² and *Xenopus laevis*³ and

mitochondrial-specific tRNA from rat liver^{4,5} have been shown to hybridize with mitochondrial DNA from the homologous source, and they are therefore presumed to be transcripts of mitochondrial DNA. Second, respiratory mutants have been described in *Saccharomyces cerevisiae*⁶⁻⁸ which are maternally inherited and seem to be the result of alterations in the amount or base composition of mitochondrial DNA. Genetic studies in yeast⁹ and *N. crassa*¹⁰⁻¹² indicate that nuclear genes are also involved in mitochondrial biogenesis. For example, the amino-acid sequence of cytochrome *c* is altered by mutations in yeast which are inherited as Mendelian characters⁹ and therefore presumably involve nuclear genes.

Two mechanisms can be envisioned for the utilization of nuclear information in mitochondrial biogenesis: messenger RNA (mRNA) could be translated by cytoplasmic ribosomes and the resultant proteins transported into the mitochondria or mRNA could pass directly from the nucleus into the mitochondria and be translated on mitochondrial ribosomes. Although there is evidence that some mitochondrial proteins

* Present address: Department of Biology, University of Virginia, Charlottesville, Virginia.

are made on cytoplasmic ribosomes¹³ it is still possible that the second mechanism is involved in the synthesis of other proteins.

Here we describe experiments which demonstrate that isolated mitochondria can incorporate high molecular weight polynucleotides which can then serve as templates for polypeptide synthesis on mitochondrial ribosomes. We suggest that a similar process may occur *in vivo* resulting in the utilization of mRNA of nuclear origin in the synthesis of mitochondrial proteins.

Stimulation by Poly U

Mitochondria obtained from *Xenopus laevis* ovaries by differential centrifugation are impermeable to pancreatic ribonuclease¹⁴. This observation formed the basis of an assay for the incorporation of polynucleotides by mitochondria. After isolated mitochondria were incubated with polyuridylic acid (poly U), ribonuclease insensitive phenylalanine incorporation into hot trichloroacetic (TCA)-insoluble material was greatly stimulated (Table 1). When ribonuclease was added to mitochondria before addition of poly U, the rate of phenylalanine incorporation was greatly reduced. In contrast, phenylalanine incorporation by the 20,000g supernatant fraction was eliminated when ribonuclease was added either before or after incubation with poly U. These results indicate that mitochondria have the ability to incorporate high molecular weight poly U which then codes for polyphenylalanine synthesis.

Swanson and Dawid reported that mitochondrial and cytoplasmic ribosomes from *X. laevis* have approximate sedimentation coefficients of 60S and 87S, respectively³. This difference made it possible to determine the type of ribosome functioning in poly U-dependent phenylalanine incorporation by the

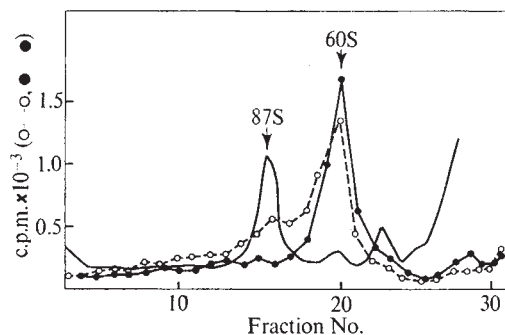


Fig. 1 Sucrose gradient fractionation of ³H-phenylalanine labelled products synthesized by mitochondria in the presence of poly U. Mitochondria were prepared from *X. laevis* ovaries using a KCl containing medium as previously described³ and the resultant mitochondrial pellet was suspended as described in Table 1. Mitochondrial suspension (1 ml.) was mixed with 25 μ l. of poly U (10 mg/ml.) and the mixture was incubated 5 min at 22° C. Aliquots (400 μ l.) were then incubated 20 min with ³H-phenylalanine in the conditions described in Table 1 (final volume of 0.5 ml.). The reaction was stopped by placing tubes in an ice bath. Mitochondria were re-isolated by centrifugation and suspended in 1.1 ml. of the homogenization medium. The non-ionic detergent NP40 (10% w/v) was added (50 μ l.), debris was removed by centrifugation and the supernate was layered on a 15–30% sucrose gradient prepared with homogenization medium. After centrifugation at 21,000 r.p.m. for 16 h at 5° C in the SW 25.3 rotor of the Beckman centrifuge, the optical density was recorded at 260 nm by pumping the gradient through a flow-through cell of the Gilford spectrophotometer. Fractions of approximately 0.5 ml. were collected, 50 μ l. of bovine serum albumin (10 mg/ml.) was added to each fraction and hot TCA-precipitable radioactivity was determined³. ○ --- ○, No additions; ● — ●, pancreatic ribonuclease added to disrupted mitochondria to a final concentration of 1 μ g/ml. before layering on the sucrose gradient; —, absorbance at 260 nm.

Table 1 Effect of Poly U on Phenylalanine Incorporation by Mitochondria

Homogenate fraction	Additions	μ mol ³ H-phenylalanine incorporated/h/mg protein
Mitochondria	Control	338.6
Mitochondria	RNAase after 1st incubation	235.9
Mitochondria	RNAase before 1st incubation	3.7
Mitochondria	Minus poly U	7.5
Post-mitochondrial supernatant	Control	33
Post-mitochondrial supernatant	RNAase after 1st incubation	0
Post-mitochondrial supernatant	RNAase before 1st incubation	0
Post-mitochondrial supernatant	Minus poly U	0.3

Whole ovaries of *X. laevis* were homogenized with a Dounce homogenizer in 5 volumes of 0.25 M sorbitol, 7 mM MgCl₂, 0.03 M Tris-HCl (pH 8) which was mixed before use with 1/9 volume 0.06 M mercaptoethanol. Mitochondria and post-mitochondrial supernatant were prepared by differential centrifugation as described previously³. Mitochondria were washed once and suspended in homogenization medium (1 ml./g ovary). Aliquots (200 μ l.) of mitochondrial suspension or post-mitochondrial supernatant were mixed with 5 μ l. of an aqueous solution of poly U (10 mg/ml.) and incubated 3 min in a water bath of 22° C. 100 μ l. aliquots were then transferred to tubes containing 100 μ l. of an assay medium constituted such that the complete system contained: 4.9 mM mercaptoethanol, 0.8 mM ATP, pyruvate kinase (78 μ g/ml.), 0.04 mM GTP, 40 mM KCl, 15 mM MgCl₂, 0.2 M sorbitol, 0.03 M Tris (pH 8) and 4 μ M ³H-phenylalanine (1.65 Ci/mmol). When ribonuclease (238 μ g/ml.) was present it was added either immediately before or after the first 3 min incubation period as indicated. Aliquots (100 μ l.) were removed before and after 1 h of incubation at 30° C. Hot TCA-precipitable radioactivity was determined as previously described³. All values were corrected for zero time controls. In this and following experiments protein concentrations were determined by the biuret method¹⁵.

mitochondrial fraction. Mitochondria were incubated first with poly U, then with ³H-phenylalanine and finally washed by centrifugation. After addition of the non-ionic detergent NP40, debris was removed by centrifugation and the distribution of hot TCA precipitable radioactivity was determined after centrifugation through sucrose gradients. Although the mitochondrial preparations were heavily contaminated by cytoplasmic 87S ribosomes, most of the radioactive material was found to sediment with the 60S mitochondrial ribosomes (Fig. 1). It seems likely that the small amount of labelled material sedimenting in the 87S region consisted of polysomal aggregates of 60S ribosomes since it was displaced to the 60S region when the disrupted mitochondria were treated with a small amount of ribonuclease (Fig. 1). Poly U-dependent phenylalanine incorporation by the mitochondrial fraction therefore seems to take place only on mitochondrial ribosomes. It is likely that the mitochondrial ribosomes are located inside the mitochondria while the cytoplasmic ribosomes are located on the outer mitochondrial membrane. This conclusion is based on the fact that most of the cytoplasmic, but not mitochondrial ribosomal, RNA is removed by treatment of mitochondria with ribonuclease or by removal of the outer mitochondrial membrane¹⁴. Because washed mitochondria were used in these experiments it is likely that there was very little soluble material outside the mitochondria. This may be the reason for the inactivity of the 87S ribosomes in the mitochondrial preparations; they did not have access to the soluble factors required for polypeptide synthesis.

In an attempt to determine whether poly U specifically stimulates phenylalanine incorporation, mitochondria were incubated with poly U and tested for ability to incorporate various labelled amino-acids in products which were insoluble in hot TCA. The results are shown in Table 2. Poly U stimulated incorporation of phenylalanine and leucine about forty-fold and four-fold, respectively, while it had no effect on the incorporation of lysine, proline or methionine. Similar

results have been obtained with subcellular preparations from bacteria and rat liver¹⁶.

Table 2 Incorporation of Amino-acids by Mitochondria

³ H-amino-acid	Additions before 1st incubation	μmol ³ H-amino-acid incorporated/h/mg protein
Phenylalanine	None	5.2
Phenylalanine	Poly U	213.6
Leucine	None	4.8
Leucine	Poly U	18.0
Lysine	None	0
Lysine	Poly U	0
Proline	None	2.3
Proline	Poly U	2.6
Methionine	None	5.8
Methionine	Poly U	5.0

Mitochondrial suspension was prepared as in Fig. 1. Mitochondrial suspension (700 μl.) was incubated with 25 μl. of poly U (10 mg/ml. water) at 22° C for 3 min and 80 μl. aliquots were then mixed with 20 μl. of an assay medium constituted such that the final concentrations of cofactors were as described in Table 1. Ribonuclease was added to all tubes at a final concentration of 238 μg/ml. ³H-amino-acids were substituted for phenylalanine as indicated and incorporation into TCA-insoluble material was assayed as previously described³.

Table 3 Stimulation of Incorporation of Amino-acids by Synthetic Polynucleotides

³ H-amino-acid	Additions before 1st incubation	μmol ³ H-amino-acid incorporated/h/mg protein
Phenylalanine	None	3.1
Phenylalanine	Poly U	233.8
Proline	None	1.2
Proline	Poly C	11.6
Leucine	None	4.8
Leucine	Poly UC	57.4
Leucine + unlabelled serine, proline, phenylalanine	None	4.7
Leucine + unlabelled serine, proline, phenylalanine	Poly UC	67.5
Proline + unlabelled serine, leucine, phenylalanine	None	1.8
Proline + unlabelled serine, leucine, phenylalanine	Poly UC	38.1

Mitochondria were prepared with a KCl medium as previously described³. The resultant pellet was suspended in the sorbitol medium described in Table 1. Aliquots (160 μl.) were each incubated with 10 μl. of an aqueous polynucleotide solution (10 mg/ml.) and assayed for amino-acid incorporation into hot TCA-insoluble material as described in Table 2. After incubation, polyproline was added to the reaction mixtures containing ³H-proline to a final concentration of 0.5 mg/ml. For precipitation of ³H-proline labelled polypeptides 20% TCA was used¹⁸.

Stimulation by Other Polynucleotides

Mitochondria were also incubated with other synthetic polynucleotides and tested for the ability to incorporate the amino-acids for which these polynucleotides have been shown to code in other subcellular systems. As Table 3 shows, each polynucleotide stimulated incorporation of the expected amino-acid¹⁷. The poly UC used was a random copolymer containing 45% uridine and 55% cytosine and was expected to code for incorporation of leucine, serine, proline and phenylalanine. On the basis of results obtained with other systems one would expect such a random copolymer to stimulate the synthesis of a TCA-insoluble polypeptide only when all amino-acids coded for by the polynucleotide are

present. Because incorporation of ³H-leucine was only slightly stimulated by the addition of the other three unlabelled amino-acids (Table 3) it seems likely that the mitochondria contained an amino-acid pool. Evidence for such a pool in both plant¹⁹ and animal²⁰ mitochondria has been reported.

Incorporation of Labelled Polynucleotides

Incorporation of polynucleotides into mitochondria was also assayed with ³H-labelled natural and synthetic RNAs. Each ³H-RNA was incubated with a suspension of mitochondria and unincorporated material was digested by further incubation with pancreatic ribonuclease. The mitochondria were filtered through glass 'Millipore' filters, washed once with cold homogenization medium and twice with cold 5% TCA. The filters were then dried and radioactivity was determined in a liquid scintillation spectrometer (Table 4). Several synthetic polynucleotides become inaccessible to ribonuclease and seem to be incorporated into mitochondria. This reaction has an absolute dependence on magnesium ions and is greatly stimulated by mercaptoethanol. No other co-factor appears to be required and although the reaction occurs only very slowly at 0° C all attempts to show a dependence on an energy source have been unsuccessful.

Table 4 Incorporation of Polynucleotides by Mitochondria

³ H-polynucleotide	μmol polynucleotide phosphorus incorporated/3 min/mg protein
Poly U	44.3
Poly C	18.5
Poly UC (1 : 1.24)	34.2
Poly AC (1 : 3.6)	19.2
Poly AU (1 : 3.3)	18.3

Mitochondrial suspension was prepared as described in Table 1. Aliquots (200 μl.) were mixed with 5 μl. of a ³H-polynucleotide solution (10 mg/ml.), the specific activities (mCi/mmol) being poly U, 7.76; poly UC, 7.55; poly AC, 7.5; and poly AU, 11.3, and incubated at 22° C for 3 min. The results of kinetic studies indicate that the reaction is linear for 3–4 min. Two volumes of pancreatic ribonuclease (1 mg/ml. of 0.1 M potassium phosphate, pH 7) were added and incubation was continued for 10 min. Each sample was then filtered through a Gelman type E glass 'Millipore' filter, washed once with 5 ml. cold homogenization medium and twice with 5 ml. cold 5% TCA. The filters were then dried and radioactivity was determined in a liquid scintillation spectrometer. Results are corrected for duplicate samples which were not incubated before addition of ribonuclease; blank values ranged from 0.75 to 4.3 μmol/mg protein.

As Table 4 shows, all polynucleotides were not incorporated to the same extent. Furthermore, there was no incorporation of either native or heat denatured nuclear DNA from *X. laevis*. These results indicate that the extent of incorporation of a polynucleotide by mitochondria is dependent on base composition.

To determine further the nature of this specificity, the effect of formaldehyde treatment of RNA on incorporation was tested. Various RNAs were labelled by incubation with ³H-dimethylsulphate²¹ and samples of each were treated with formaldehyde²² thereby destroying secondary structure as determined by hyperchromicity measurements. The extent of incorporation of the treated and untreated samples was then measured. The results in Table 5 indicate that very little untreated ribosomal RNA (rRNA) or tRNA was incorporated. Although formaldehyde treatment increased the incorporation of poly C, tRNA and rRNA, the level of incorporation of each of these polynucleotides was still much less than that of poly U. These results suggest that the secondary structure of a polynucleotide plays only a small part in determination of specificity in the incorporation reaction.

The observation that formaldehyde treatment did not alter poly U incorporation is in agreement with the fact that formaldehyde reacts only with the primary amino groups of guanine, cytosine and adenine^{23,24}.

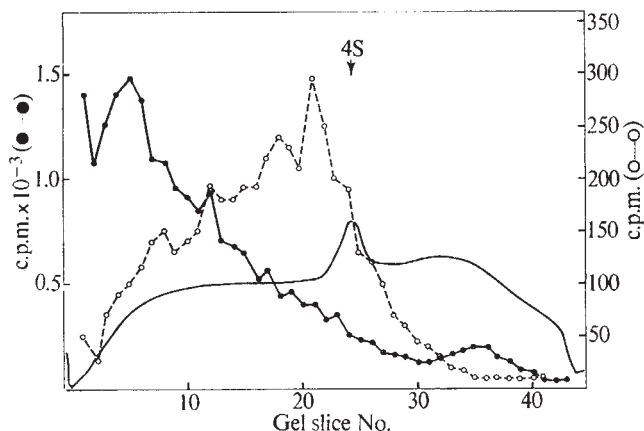


Fig. 2 Electrophoresis of ³H-poly U re-isolated after incorporation by mitochondria. Mitochondrial suspension was prepared as described in Table 1. A sample (1 ml.) of the suspension was incubated for 3 min with 25 μ l. of ³H-poly U (10 mg/ml.) at 22° C and treated with ribonuclease as described in Table 4. Mitochondria were re-isolated by centrifugation, RNA was extracted with phenol and sodium dodecyl sulphate (SDS) and analysed by electrophoresis on 9% polyacrylamide gels in the presence of SDS²³. The direction of migration is from left to right; that is, toward the anode. The gels were scanned at 260 nm with a Joyce Chromoscan and then sliced into 2 mm sections. Each section was incubated with 0.5 ml. 0.3 N KOH for 16 h at 37° C in a scintillation vial. Scintillation fluid (10 ml.) was added to each vial and radioactivity was determined in a liquid scintillation spectrometer. As controls, untreated ³H-poly U and yeast tRNA were each subjected to electrophoresis and distribution of radioactivity and ultra-violet absorbing material in the gels was determined in the same way. ●—●, Untreated ³H-poly U; ○—○, ³H-poly U extracted from mitochondria; - - -, OD260 of the gel containing yeast tRNA.

A comparison of Tables 4 and 5 shows that the extent of incorporation of poly U varies with different mitochondrial preparations. It has been found, however, that the relative rates of incorporation of polynucleotides by a single preparation are always about the same.

Size of Incorporated Poly U

The results in Table 5 suggest that differences in mitochondrial permeability to various polynucleotides were not caused by selective incorporation of only low molecular weight RNAs, for tRNA was incorporated to a much smaller extent than were the much larger synthetic polynucleotides. This latter statement is based on the assumption that the molecular weights of the RNAs were not drastically changed as a result of incubation with mitochondria. The validity of this assumption was tested by extracting incorporated ³H-poly U from mitochondria and analysing it by electrophoresis on polyacrylamide gels²⁵. As Fig. 2 shows, incorporated poly U was partly degraded. It is important, however, that the molecular size of most of the incorporated material was still at least as large as that of tRNA; that is, very little of the incorporated poly U migrated more rapidly than tRNA. Therefore, molecular size does not seem to determine specificity.

In conclusion, intact mitochondria were penetrated by specific polynucleotides which then served as templates for polypeptide synthesis on mitochondrial ribosomes. The fact that the specificity of the incorporation reaction was dependent at least in part on polynucleotide base composition suggests

that this reaction is more than a non-specific diffusion process into damaged mitochondria. Furthermore, the mere existence of specificity in this reaction suggests that it occurs *in vivo* and that messenger RNA of nuclear origin is used in mitochondrial protein synthesis.

Table 5 Effect of Formaldehyde on Incorporation of Polynucleotides by Mitochondria

³ H-polynucleotide	Treatment of polynucleotide	μ mol of polynucleotide phosphorus incorporated/3 min/mg protein
Poly U	None	14.0
Poly U	Formaldehyde	13.9
tRNA	None	0.4
tRNA	Formaldehyde	1.0
Poly C	None	3.3
Poly C	Formaldehyde	5.3
rRNA	None	0.1
rRNA	Formaldehyde	0.5

Yeast tRNA, *X. laevis* rRNA and poly C were each labelled chemically with ³H-dimethylsulphate¹⁹. Aliquots were treated with formaldehyde²⁰ which was then removed by repeated precipitation with ethanol. Mitochondrial suspensions were prepared as in Table 1 and the various polynucleotides were tested for ability to be incorporated by mitochondria as described in Table 4.

This work was supported by a US Public Health Service research fellowship. I thank Drs Rolf Benzinger, Igor Dawid, Charles Emerson, Elizabeth Kutter and Robert Kretsinger for reviewing my manuscript. I also thank Dr Igor Dawid for helpful advice and discussion.

Received December 21, 1970; revised February 16, 1971.

- Nass, M. M. K., *Science*, **165**, 25 (1969).
- Wood, D. D., and Luck, D. J. L., *J. Mol. Biol.*, **41**, 211 (1969).
- Swanson, R. F., and Dawid, I. B., *Proc. US Nat. Acad. Sci.*, **66**, 117 (1970).
- Buck, C. A., and Nass, M. M. K., *J. Mol. Biol.*, **41**, 67 (1969).
- Nass, M. M. K., and Buck, C. A., *Proc. US Nat. Acad. Sci.*, **62**, 506 (1969).
- Mounolou, J. C., Jacob, H., and Slonimski, P. P., *Biochem. Biophys. Res. Commun.*, **24**, 218 (1966).
- Moustacchi, E., and Williamson, D. H., *Biochem. Biophys. Res. Commun.*, **23**, 56 (1966).
- Tewari, K. K., Vötsch, W., Mahler, H. R., and Mackler, B., *J. Mol. Biol.*, **20**, 453 (1966).
- Sherman, F., Stewart, J. W., Margoliash, E., Parker, J., and Campbell, W., *Proc. US Nat. Acad. Sci.*, **55**, 1498 (1966).
- Mitchell, H. K., and Mitchell, M. B., *J. Gen. Microbiol.*, **14**, 184 (1956).
- Munkres, K. D., Giles, N. H., and Case, M. E., *Arch. Biochem. Biophys.*, **109**, 397 (1965).
- Munkres, K. D., and Richards, F. M., *Arch. Biochem. Biophys.*, **109**, 457 (1965).
- Ashwell, M., and Work, T. S., in *Annual Review of Biochemistry* (edit. by Snell, E. E.), **39**, 251 (Annual Reviews Inc., 1970).
- Dawid, I. B., in *The Control of Organelle Development* (edit. by Miller, P.), *Symp. Soc. Exp. Biol.*, **24**, 227 (Cambridge University Press, 1970).
- Layne, E., *Methods in Enzymology*, **3** (1957).
- Weinstein, I. B., Friedman, S. M., and Ochoa, M., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 671 (1966).
- Bretscher, M. S., and Jones, O. W., in *Techniques in Protein Biosynthesis* (edit. by Campbell, P. N., and Sargent, J. R.), **1**, 216 (Academic Press, 1967).
- Wahba, A. J., Gardner, R. S., Basilio, C., Miller, R. S., Spegard, F., and Lengyel, P., *Proc. US Nat. Acad. Sci.*, **49**, 116 (1963).
- Das, H. K., Chatterjee, S. K., and Roy, S. C., *J. Biol. Chem.*, **239**, 1126 (1964).
- Beattie, D. S., Basford, R. E., and Koritz, S. B., *Biochemistry*, **6**, 3099 (1967).
- Smith, K. D., Armstrong, J. L., and McCarthy, B. J., *Biochim. Biophys. Acta*, **142**, 323 (1967).
- Boedtker, H., *Biochemistry*, **6**, 2718 (1967).
- Fraenkel-Conrat, H., *Biochim. Biophys. Acta*, **15**, 307 (1954).
- Staehelin, M., *Biochim. Biophys. Acta*, **29**, 410 (1958).
- Weinberg, R. A., Loening, U., Willems, M., and Penman, S., *Proc. US Nat. Acad. Sci.*, **58**, 1088 (1967).

LETTERS TO NATURE

PHYSICAL SCIENCES

Radio Spectrum of Maffei 2

THE conclusion that Maffei 1 and 2 are previously unrecognized nearby galaxies¹ has aroused considerable interest; a positive detection of high frequency radio emission from Maffei 2 has been reported^{2,3} as also has a null result in the direction of Maffei 1 (ref. 4). Here we report observations at a much lower frequency; they are results obtained during a radio survey⁵ using the Cambridge 178 MHz pencil-beam synthesis instrument, with beam size $18'.5 \times 23'.4$.

A source with flux density 4.2 ± 1.0 f.u. (1 f.u. = 10^{-26} W m⁻² Hz⁻¹) was detected at right ascension 02 h 38 m 10 s, declension $+59^\circ 20'.5$ (1950), which is $3'$ south of the centre of Maffei 2. This difference is larger than our usual standard error in declination ($1'.2$) because the measurement is affected by the proximity of 3C 69; the source is definitely the same as that detected at higher frequencies and identified with Maffei 2. The recent measurement³ at 1,415 MHz gave a flux density of 1.08 ± 0.10 f.u. for Maffei 2 which, combined with our value of 4.2 f.u. at 178 MHz, yields a spectral index, α , of 0.65 ± 0.1 (where α is defined by flux density $\propto$ (frequency)^{- α}). Bell *et al.*² derived a spectral index of 1.30 ± 0.05 between 10,700 and 3,200 MHz but such a steep spectrum certainly does not continue to low frequencies as a flux density of 20 f.u. at 178 MHz would then be expected. Thus, the spectrum seems to steepen at high frequency, and De Jong⁶ finds evidence of a similar change of slope in the spectra of some other normal galaxies at more than 1,400 MHz. But the available angular size data suggest that the steepness of the high frequency spectrum of Maffei 2 may have been overestimated. The 1,415 MHz measurements³ indicate a source width (in right ascension) of $3'.4 \pm 0'.5$, whereas Bell *et al.*² detected no broadening and consequently their flux densities are peak values, not corrected for the finite size of the source. Any flux density correction factors, allowing for finite source size, would be larger at higher frequencies and produce a spectrum flatter than before correction. For example, if the intrinsic source size between 3,200 and 10,700 MHz were $2'$ (the upper limit derived by Bell *et al.*²), the spectral index in this frequency range would be 1.05; if the source size were $3'.4$ (using the 1,415 MHz measurement in preference to the higher frequency $2'$ upper limit), the spectral index would be 0.75 and would show scarcely any change over the entire range 178–10,700 MHz.

The search⁴ at 1,415 MHz for a source associated with Maffei 1 revealed a previously uncatalogued radio source $\approx 15'$ north of Maffei 1, with flux density 0.427 ± 0.001 f.u. This source is also present on the 178 MHz records in which it has a flux density of 2.5 ± 1 f.u. It therefore seems to be non-thermal,

with spectral index ≈ 0.9 , but its separation from the centre of Maffei 1 suggests it is an unrelated background source.

J. L. CASWELL

Division of Radiophysics,
CSIRO, Sydney

Received April 13, 1971.

- ¹ Spinrad, H., Sargent, W. L. W., Oke, J. B., Neugebauer, G., Landau, R., King, I. R., Gunn, J. E., Garmire, G., and Dieter, N. H., *Astrophys. J. Lett.*, **163**, L25 (1971).
- ² Bell, M. B., Seaquist, E. R., and Braun, L. D., *Astrophys. J. Lett.*, **161**, L13 (1970).
- ³ Bottinelli, L., Chamaraux, P., Gérard, E., Gouguenheim, L., Heidmann, J., Kazès, I., and Lauqué, R., *Astron. Astrophys.* (in the press).
- ⁴ Oort, J. H., *Nature*, **230**, 103 (1971).
- ⁵ Caswell, J. L., and Crowther, J. H., *Mon. Not. Roy. Astron. Soc.*, **145**, 181 (1969).
- ⁶ De Jong, M. L., *Astrophys. J.*, **150**, 1 (1967).

Are the Maffei Galaxies Members of the Local Group?

I SUGGEST in this report that the highly obscured galaxy IC342 might be associated with Maffei 1 and Maffei 2 (refs. 1 and 2). IC342 is the fourth largest (after M31, M33 and M101) spiral galaxy known. It is separated from Maffei 1 by less than 12° which, at a distance of 2 M parsec (Mpc), would correspond to a projected separation of only 0.4 Mpc. Available data on Maffei 1 and on IC342 are compared in Table 1.

Table 1 Data on Maffei 1 and IC342

Object	l''	b''	V^* (km s ⁻¹)	Distance (Mpc)
Maffei 1	135.8°	-0.6°	$+188^\dagger$	$0.5 \leq D \leq 2$
IC342	138.2°	$+10.6^\circ$	$+195^\ddagger$	$D \approx 2$

* Radial velocity relative to the Local Group (see de Vaucouleurs and de Vaucouleurs⁸). † From Spinrad *et al.*². ‡ From Ford *et al.*⁷.

IC342 has been suggested^{3,4} to be a possible Local Group member. Recent investigations indicate that IC342 is probably too distant to be considered a *bona fide* member of the Local Group. From 21 cm observations Dieter⁵ obtained a distance

$$D \text{ (Mpc)} = 18 \mathcal{M}(\text{HI}) / \mathcal{M}(\text{total}) \quad (1)$$

Substituting the value ($\mathcal{M}(\text{HI}) / \mathcal{M}(\text{total})$) = 0.10, that Roberts⁶ obtained for galaxies of type Sc, into equation (1) yields a distance of 1.8 Mpc. This value is probably uncertain by about a factor of two. Summarizing available optical data, de Vaucouleurs (quoted by Dieter⁵) concluded that IC342 is

located at a distance of 2.2 ± 0.4 Mpc. A value $D \approx 2$ Mpc is not inconsistent with the distance estimate $0.5 \leq D$ (Mpc) ≤ 2.0 that Spinrad *et al.*² obtained for Maffei 1. It should, however, be emphasized that a distance of about 2 Mpc would place the Maffei objects somewhat beyond the generally accepted limits of the Local Group^{9,10}.

SIDNEY VAN DEN BERGH

David Dunlap Observatory,
University of Toronto,
Richmond Hill, Ontario

Received April 13, 1971.

- ¹ Maffei, P., *Pub. Astron. Soc. Pacific*, **80**, 618 (1968).
- ² Spinrad, H., Sargent, W. L. W., Oke, J. B., Neugebauer, G., Landau, R., King, I. R., Gunn, J. E., Garmire, G., and Dieter, N. H., *Astrophys. J. Lett.*, **163**, L25 (1971).
- ³ Shapley, H., and Seyfert, C. K., *Harvard Obs. Bull.*, No. 899 (1935).
- ⁴ Hubble, E., *The Realm of the Nebulae*, 125 (Yale University Press, New Haven, 1936).
- ⁵ Dieter, N. H., *Astron. J.*, **67**, 313 (1962).
- ⁶ Roberts, M. S., *Astron. J.*, **74**, 859 (1969).
- ⁷ Ford, W. K., Rubin, V. C., and Roberts, M. S., *Astron. J.*, **76**, 22 (1971).
- ⁸ de Vaucouleurs, G., and de Vaucouleurs, A., *Reference Catalogue of Bright Galaxies*, 3 (University of Texas Press, Austin, 1964).
- ⁹ Bergh, S. van den, *J. Roy. Astron. Soc. Canada*, **62**, 145 (1968).
- ¹⁰ Bergh, S. van den, *J. Roy. Astron. Soc. Canada*, **62**, 219 (1968).

Radio Continuum Observations of Maffei 2

WE have mapped the area containing the suspected galaxies Maffei 1 and 2 at a wavelength of 11 cm with half-power beam-width 5'.0 using the 300 foot transit telescope of the National Radio Astronomy Observatory at Green Bank, West Virginia, from January 28 to 31, 1971. A three-feed system with four receivers was used; the centre feed provided independent right- and left-hand circularly polarized beams at the same point in the sky, and two outrigger beams (2' north and 2' south of the centre beam) each received right hand circularly polarized radiation. Several transits with the telescope set at different declinations provided full mapping of an area between right ascensions 02 h 25 m and 02 h 43 m (1950.0) and between declinations $+59^\circ 15'$ and $+59^\circ 33'$ (1950.0). The output of the radiometers was recorded every 4 s. With the parametric amplifiers used, the lower flux density limit was set by confusion rather than by system noise. We estimate that the lower flux density limit for detection of a point source is 0.06 f.u.

No emission was detected from the suspected position of Maffei 1, which agrees with the findings of Bell *et al.*¹ and of Oort².

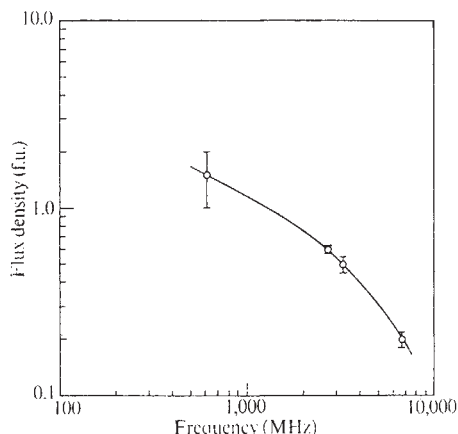


Fig. 1 The spectrum of the small-diameter radio source associated with Maffei 2 between 4.5 and 49 cm wavelength.

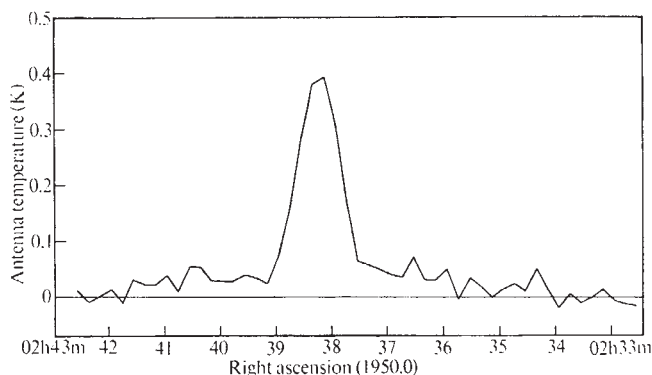


Fig. 2 A drift scan across Maffei 2 at $\delta = +59^\circ 24' 35''$ and at 11 cm wavelength.

At the position $\alpha = 02 \text{ h } 38 \text{ m } 12.3 \text{ s} \pm 1 \text{ s}$, $\delta = +59^\circ 23' 03'' \pm 30''$, we have detected the small diameter source found by Bell *et al.* We found that it was unresolved with our beam, and had flux density 0.60 ± 0.03 f.u. This is consistent with the findings of Bell *et al.* at 9.26 and 4.5 cm. We found that the spectrum from these three points is adequately described by a straight line in a $\log S_\nu - \log \nu$ plot, with spectral index $= -1.21 \pm 0.12$. We have also examined records at a wavelength of 49 cm from the 400-foot telescope of the Vermilion River Observatory of the University of Illinois. This region of the galactic plane has already been mapped at this wavelength³; the area around Maffei 2 appears as a slight increase in antenna temperature on their contour map. We found that there is a source possibly larger than $16'$ of arc in right ascension at the position of Maffei 2, with a flux density of 1.5 ± 0.5 f.u. This indicates a spectrum which decreases at short wavelengths, as shown in Fig. 1, which is not unusual for ordinary extragalactic sources.

As well as the small-diameter source, we have detected at 11 cm a very weak, extended source surrounding it. Fig. 2 shows the average of the two central beams at a declination of $+59^\circ 24' 35''$, slightly north of the centre of the source. The data have been smoothed to an effective integration time of 12 s per point. The area between about 02 h 35 m and 02 h 42 m is higher than the background by about 0.05 K of antenna temperature (the scale for point sources at this declination being 1 K = 1.00 f.u.). This extended region also appears on tracings at adjacent declinations, and may extend about $8'$ in declination and $30'$ in right ascension. The integrated flux density of the extended region is thus approximately 0.5 f.u.

Because this region is in the plane of our galaxy, the extended component may merely be an increase in the background radiation, or some weak source within the galaxy. But our map of the larger surrounding area shows no other extended region of comparable size and antenna temperature, and we believe that there is a significant chance that the extended region is associated with Maffei 2. At a nominal distance of 1 Mpc, the linear dimensions of the extended source would be about 2,300 by 8,800 Mpc, not unreasonable numbers for the denser inner regions of a spiral galaxy, tilted by about 75° .

This work was supported by a grant from the US National Science Foundation. The National Radio Astronomy Observatory is operated by Associated Universities, Inc., for the National Science Foundation.

J. C. WEBBER
A. G. WILLIS

University of Illinois Observatory,
Urbana, Illinois 61801

Received April 5, 1971.

- ¹ Bell, M. B., Seaquist, E. R., and Braun, L. D., *Astrophys. J. Lett.*, **161**, L13 (1970).
- ² Oort, J. H., *Nature*, **230**, 103 (1971).
- ³ Webb, H. D., and Dickel, J. R., *Astron. J.* (in the press, 1971).

Hydrographic Observations on the Red Sea Brines indicate a Marked Increase in Temperature

THE bathymetric and hydrographic data from the expedition of the R/V Chain during November 1966 revealed three deeps in the Red Sea all containing hot brine¹. The largest of these, the Atlantis II deep, contained bottom water at 56.5° C and with salinity 257‰, above which was an intermediate layer at 44.3° C and salinity 135‰. The two adjacent deeps (Discovery deep and Chain deep) contain brine at a lower temperature and it has been suggested that these represent spillover from the active site¹.

During February 1971, R/V Chain revisited the brine area. Although it will be several months before the chemical data are complete, the hydrographic data gathered are of immediate interest. The most unusual observation is that the temperature of the Atlantis II brine has risen 2.7° C from 56.5° C to 59.2° C in the 51 months since the last published observation. Munns *et al.*² had noted an apparent increase in temperature of 0.56° C in the 20 months between February 1965 and October 1966, but that important observation was not conclusive evidence of brine activity because of the uncertainties in using oceanographic thermometers outside their normal range. For the 1971 cruise, special high range protected thermometers were used, and it is estimated that these observations were accurate to about $\pm 0.05^\circ \text{C}$ (Fig. 1). The interface between the 59° C brine and the intermediate brine seems to have risen by about 6 m since 1966, giving an increase in volume of this brine of about 12%. No significant change was detected in the salinity of the deep Atlantis II brine, but it should be noted that the salinities reported here are conductometric values obtained by volume dilution of the sample. The systematic errors introduced by this procedure are such that in these waters a conductometric salinity of 320‰ is equal to a true gravimetric salinity of 257‰ (ref. 3).

Since 1966, the intermediate layer above the 59° C brine has also increased in temperature by 5.4° C, from 44.3° C to 49.7° C. The conductometric salinity of this layer has increased from 151.5–154‰, an increase in true (gravimetric) salinity of 2.2‰. Although the topmost brine interface has risen about 6 m (Fig. 1), only the lower brine mass has significantly increased in volume. The marked temperature increase and small salinity increase indicate that heat transfer across the lower interface has occurred primarily by diffusion, rather than by turbulent mixing. But, because strong unstable salinity (T. R. S. W., unpublished data) and temperature (D. A. Ross, private communication) layering have now been detected within both the upper and lower interfaces, more active transport processes may have become important very recently.

Observations made in the Discovery deep did not reveal any changes in brine temperature or salinity since 1966. It has generally been assumed that this brine is renewed by overspill from the Atlantis II deep, in which case the recent activity in this deep has not yet caused such an overflow, a conclusion supported by the relatively small changes observed in the depth of the Atlantis II intermediate layer. Although it has been suggested³ that there may be a steady loss in volume of the Discovery brines, the interface depth observed during the present cruise is within the range of previous observations.

Ross and Hunt⁴ reported a third deep (the Chain deep) containing hot brine on the saddle between the Atlantis II and Discovery deeps. In 1966, one hydrocast in this narrow and steep-sided deep revealed a temperature of 29.07° C and a salinity of 74.2‰. Attempts to place a hydrocast in the Chain deep on this cruise failed because of high winds and consequent ship drift.

The 1966 bathymetric chart⁵ revealed a long, narrow depression to the north-east of the Atlantis II deep, which has not previously been sampled. On this cruise two stations (Nos. 1171 and 1172) were made in the new deep, which was

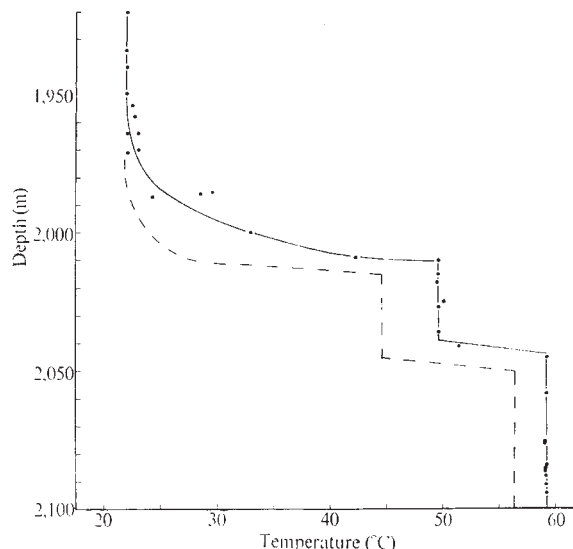


Fig. 1 Temperature profile through Atlantis II brine layers, February 1971. Dashed line represents 1966 profile³.

found to contain hot brine. The maximum temperature recorded was 56.69° C (conductometric salinity, 318‰) and above this brine was a layer at 49.9° C (conductometric salinity, 209‰). It seems possible that this brine originates in the Atlantis II deep; but the high temperature and the existence of a sill shallower than 1,900 m between the deeps make this by no means certain, and the deep could represent a new active source.

Swallow⁶ has described a reflecting layer, seen on the echo sounder of RRS Discovery, in a deep close to 21° 08' N, 38° 06' E, which may also contain hot brine. Our survey revealed a large depression and a hydrocast was made in a depth of 2,465 m (corrected). A bottom temperature of 22.17° C and salinity of 40.99‰ were recorded; although this is slightly anomalous, it is not evidence for a new brine deep. The reflecting layer reported by Swallow lay some 21 m below this; our findings do not, therefore, exclude the possibility of a thin brine layer in a deeper part of this basin.

The important discoveries of this cruise are, therefore, the increased temperature of the Atlantis II deep, the lack of a corresponding change in the Discovery deep, and the finding of a new brine hole north-east of the Atlantis II deep. From the data obtained it is possible to outline the process which has caused the changes since 1966.

The hydrographic observations (Fig. 1) show an increase in the temperature and thickness of the lowest layer of the Atlantis II deep, although the intermediate layer has changed little in volume or salinity. This implies water input of 257‰ (gravimetric) salinity at some temperature (t) higher than the present temperature of this layer, followed by a largely diffusive flow of heat to the intermediate layer. If it is assumed that the brines have lost no heat to the surroundings since this inflow of hot brine, the minimum temperature of the inflow can be calculated. From the dimensions of the brine layers⁷ and brine density data³ the ratio

$$\left(\frac{M_2}{M_1} \right)$$

of the mass of the intermediate layer (2) to that of the deep layer (1) as 0.65 is obtained. No data exist on the specific heat (C_p) of the brines. Adequate estimates can be made by extrapolation from the results of Gucker and Rubin⁸ for sodium chloride solutions, and those of Bromley *et al.*⁹ for seawater. The estimated values are 0.86 for the 135 ml.⁻¹ brine, and 0.80

for the 257 ml.⁻¹ brine. Because

$$C_{p1} M_1 \theta_1 + M_2 \theta_2 C_{p2} + \frac{x}{L} M_1 t C_{p1} = \frac{(L+x)}{L} M_1 \theta_1' C_{p1} + M_2 \theta_2' C_{p2}$$

(where M_1 , M_2 are the masses of the appropriate brine layer; θ and θ' the initial and final temperatures; L the original mean depth of layer 1, and x the increase in depth of this layer since 1966 (Fig. 1)), we can estimate the minimum original temperature of the input brine as 113° C.

The actual temperature of the "new" water must have been higher than this, as there must have been some heat loss to the surrounding sediments and the Red Sea bottom water. Uncertainties in the rates of heating and heat transport render quantitative evaluation of these losses impossible. Rough estimates, based on available heat loss data^{10,11}, indicate that the input brine temperature is probably substantially below the boiling point of water (360° C) at the ambient pressure of 200 bars.

Table 1 Dissolved Iron and Manganese Values from Atlantis II Deep

Cruise	Analysis	
	Fe (g kg ⁻¹)	Mn (g kg ⁻¹)
Atlantis II 1965	6×10^{-2}	7×10^{-2}
Chain 1966	8.1×10^{-2}	8.2×10^{-2}
Chain 1971	9.5×10^{-2}	9.8×10^{-2}

This concept is supported by preliminary analyses of both iron and manganese. (These data are compared with previous analyses in Table 1.) It is clear that a steady increase in both constituents has occurred simultaneously with the observed temperature increase. In the conditions in the body of the lowest brine layer, both iron and manganese remain in solution; their rate of removal by precipitation at the interface is controlled by the rate at which oxygen and carbon dioxide diffuse down into this layer. This oxidation and precipitation is the principal mechanism of formation of the mineral-rich sediments of this basin. The observed increase in dissolved iron and manganese therefore indicates that over the period of observation, the rate of input of these elements in the reduced form has greatly exceeded the rate of deposition of the oxidized form. These observations indicate a system in which ore formation is occurring, rather than a relict system decaying from a previously active phase.

We thank Susan Kadar and Edith Ross for help on the cruise and the officers and crew of RV Chain for their excellent cooperation. This work was supported by a grant from the US National Science Foundation.

P. G. BREWER
T. R. S. WILSON
J. W. MURRAY
R. G. MUNNS
C. D. DENSMORE

Woods Hole Oceanographic Institution,
Woods Hole, Massachusetts 02543

Received April 13, 1971.

¹ Degens, E. T., and Ross, D. A., *Hot Brines and Recent Heavy Metal Deposits in the Red Sea* (edit. by Degens, E. T., and Ross, D. A.), 600 (Springer, New York, 1969).

² Munns, R. G., Stanley, R. J., and Densmore, C. D., *Nature*, **214**, 1215 (1967).

³ Brewer, P. G., Densmore, C. D., Munns, R. G., and Stanley, R. J., in *Hot Brines and Recent Heavy Metal Deposits in the Red Sea* (edit. by Degens, E. T., and Ross, D. A.), 138 (Springer, New York, 1969).

⁴ Ross, D. A., and Hunt, J. M., *Nature*, **213**, 687 (1967).

⁵ Ross, D. A., Hays, E. E., and Allstrom, F. C., in *Hot Brines and Recent Heavy Metal Deposits in the Red Sea* (edit. by Degens, E. T., and Ross, D. A.), 83 (Springer, New York, 1969).

⁶ Swallow, J. C., in *Hot Brines and Recent Heavy Metal Deposits in the Red Sea* (edit. by Degens, E. T., and Ross, D. A.), 3 (Springer, New York, 1969).

⁷ Ross, D. A., in *Hot Brines and Recent Heavy Metal Deposits in the Red Sea* (edit. by Degens, E. T., and Ross, D. A.), 148 (Springer, New York, 1969).

⁸ Gucker, jun., F. T., and Rubin, T. R., *J. Amer. Chem. Soc.*, **57**, 78 (1935).

⁹ Bromley, L. A., Gillan, W. S., and Coley, F. H., *J. Chem. Eng. Data*, **12**, 202 (1967).

¹⁰ Erickson, A. J., and Simmons, G., in *Hot Brines and Recent Heavy Metal Deposits in the Red Sea* (edit. by Degens, E. T., and Ross, D. A.), 114 (Springer, New York, 1969).

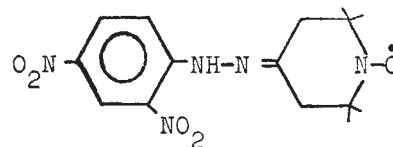
¹¹ Pugh, D. T., in *Hot Brines and Recent Heavy Metal Deposits in the Red Sea* (edit. by Degens, E. T., and Ross, D. A.), 158 (Springer, New York, 1969).

¹² Miller, A. R., Densmore, C. D., Degens, E. T., Hathaway, jun., C., Manheim, F. G., McFarlin, P. F., Pocklington, R., and Jokela, A., *Geochim. Cosmochim. Acta*, **30**, 341 (1966).

¹³ Brewer, P. G., and Spencer, D. W., in *Hot Brines and Recent Heavy Metal Deposits in the Red Sea* (edit. by Degens, E. T., and Ross, D. A.), 174 (Springer, New York, 1969).

Electron Spin Resonance Studies of the Solubilization of Nitroxide Spin Probes by Micellar Solutions

CONSIDERABLE interest has arisen recently concerning the use of nitroxide spin probes to investigate the properties of micellar solutions¹⁻⁴. Using a range of spin probes and in particular (I), Waggoner *et al.*¹⁻³ were able to test, and subsequently reject, three common static models for solubilization in favour of a dynamic model, in which the neutral probes when solubilized occupy a relatively polar environment and tumble at a rate much faster than that of a rigid micelle. Waggoner's calculations were based on the procedure of McConnell⁵ and assumed that the restriction on molecular tumbling of the probe which occurred after association with the micelles was responsible for the broadening of the electron spin resonance (ESR) spectra and excluded consideration of the exchange of solubilized spin probe between bulk water and micellar aggregates.



(I)

I intend here to draw attention to the fact that the interpretation of the ESR spectra of spin probes in micellar and related membrane-like systems is more complicated than has been assumed so far. The reasons for this are as follows. (1) The distribution of the spin probe between micellar aggregates and aqueous solution deviates considerably from a simple two-phase distribution law, significant concentrations being detected in the aqueous phase. In normal conditions, the concentrations of (I) in the two phases are comparable. (2) The rate of exchange of the spin probe between micellar and aqueous environments is slow ($< 10^6$ s⁻¹) compared with the ESR time scale. Consequently, the recorded ESR spectrum consists of a superposition of two individual spectra, above the surfactant critical micelle concentration (CMC), weighted according to the respective concentrations in aqueous and micellar states. This result accounts for the shape of the curve for the correlation time against concentration of sodium dodecyl sulphate (SDS), which has been discussed previously⁴. The exchange rate is consistent with that obtained from solubilized biradicals in SDS solutions⁶. (3) The spectral

parameters of the spin probe are similar in each environment so that resolution is not achieved, which leads to pitfalls in the interpretation of the ESR spectra and incorrect numerical assignments.

These complications have been found to be important for other spin probes such as 2,2,6,6-tetra methyl piperidine-1-oxyl and its derivatives in micellar solutions and they can also exist in mesomorphic phase systems. The complexity of the ESR spectrum depends on the solubility of the spin probe in the aqueous phase and the position which the solubilized molecule takes up in the micelle or membrane.

The presence of two superimposed ESR spectra can be demonstrated by the following. (1) Asymmetry in the recorded spectrum (Fig. 1a) and careful examination of line-shapes at surfactant concentrations above the CMC. (2) Broadening of the spectrum of the spin probe in the micellar phase (Fig. 1b) when two or more spin probes occupy the same micelle. Spin-spin broadening facilitates partial resolution of the high field line of the recorded spectrum. The other hyperfine components of the two spectra are less resolved because of the higher g -value of the spectrum of the solubilized spin probe. (3) Doping the solution with paramagnetic ions, such as Mn^{2+} or Cu^{2+} , which preferentially bind to the micellar surface.

As the last experiment can also be used to measure the affinity of ions for colloidal surfaces, it is described in more detail. The line-broadening of the peaks of the ESR spectrum of (I) in Mn^{2+} doped SDS solutions as a function of cation concentration can be described by an equation of the form^{7,8}

$$\left(\frac{1}{NT_2}\right) = \frac{4}{60} S(S+1) P \left(\frac{g^2 \beta^2 \gamma^2 e}{r^6} \right) \left[7\tau_c + \frac{13\tau_c}{1 + \omega_s^2 \tau_c^2} \right] + \frac{1}{3} S(S+1) P \left(\frac{A^2}{\hbar^2} \right) \left[\tau_c + \frac{\tau_c}{1 + \omega_s^2 \tau_c^2} \right]$$

in which the first term represents the dipole-dipole interaction and the second the spin exchange contribution to the relaxation rate. Because the spin exchange term is negligible in this system, the equation predicts that line broadening should be proportional to the cationic concentration, N , and the average distance, r , between Mn^{2+} and (I). P represents the fraction of (I) interacting with the cation, which is constant in dilute solutions, and similarly τ_c which consists of contributions

$$\frac{1}{\tau_c} = \frac{1}{\tau_s} + \frac{1}{\tau_h} + \frac{1}{\tau_r}$$

in which the first two terms have their usual significance^{7,8} but τ_r here represents the correlation time for random Brownian motion of the Mn^{2+} ion relative to that of (I).

Consequently, the two spectra of (I) in micellar solutions will be discriminately broadened as the Mn^{2+} ion concentration is increased, because Mn^{2+} tends to be absorbed preferentially into the Stern layer of the micelle in SDS solutions⁹, which results in greater proximity of Mn^{2+} and (I) solubilized in the micelle. Experimentally, a spectrum resembling that in Fig. 2 is observed in 0.5% SDS solution containing 10^{-4} M Mn^{2+} and at 10^{-3} M Mn^{2+} there is considerable reduction in signal amplitude and the spectrum of (I) in water remains. This is because the spectrum of solubilized (I) has been broadened beyond detection as a result of spin-spin interactions with Mn^{2+} ions absorbed in the Stern layer of the micelle. Finally, at 10^{-2} M Mn^{2+} the aqueous spectrum becomes severely broadened, indicating the existence of "free" Mn^{2+} ions in the aqueous phase.

In conclusion, I emphasize that care must be taken in the interpretation of the ESR spectra of spin probes in micellar and related systems, as the recorded spectrum consists of a superposition of two spectra which is not obvious. I have demonstrated how doping with Mn^{2+} helps to resolve this difficulty.

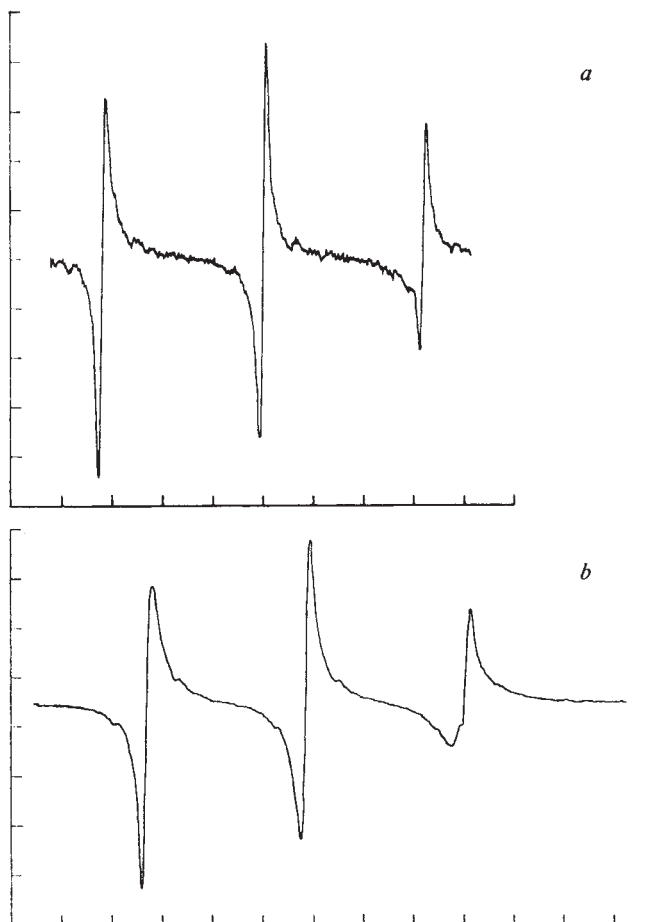


Fig. 1 a, Electron spin resonance spectrum of 10^{-5} M (I) in 0.3% SDS solutions. b, Electron spin resonance spectrum of 1.3×10^{-4} (I) in 0.5% SDS solutions. The solutions were saturated with spin probe and the average number of molecules of (I) per micelle was ~ 2 .

I thank Mr M. Barratt for a gift of (I), Mr M. C. Cafe for experimental assistance, my colleagues for helpful discussions and Dr Eastmond of Liverpool University for the use of the Varian E-3.

J. OAKES

Unilever Research Port Sunlight Laboratory,
Port Sunlight,
Wirral, Cheshire

Received November 27, 1970; revised March 18, 1971.

- Waggoner, A. S., Griffith, O. H., and Christensen, C. R., *Proc. US Nat. Acad. Sci.*, **57**, 1198 (1967).
- Waggoner, A. S., Keith, A. D., and Griffith, O. H., *J. Phys. Chem.*, **72**, 4129 (1968).
- Griffith, O. H., and Waggoner, A. S., *Accounts. Chem. Res.*, **2**, 17 (1969).
- Rabold, G. P., *J. Polymer Sci.*, A-1, **7**, 1187 (1969).
- Stone, T. J., Buckman, T., Nordio, P. L., and McConnell, H. M., *Proc. US Nat. Acad. Sci.*, **54**, 1010 (1965).
- Ohnishi, S., Cyr, T. J. R., and Fukushima, H., *Bull. Chem. Soc. Japan*, **43**, 673 (1970).
- Solomon, I., *Phys. Rev.*, **99**, 559 (1955).
- Bloembergen, N., *J. Chem. Phys.*, **27**, 572, 595 (1957).
- Pearson, J. T., and Lawrence, A. S. C., *Trans. Faraday Soc.*, **63**, 488 (1967).

A Cappadocian Speculation

IN 1925, F. Hrozny discovered the source of the "Cappadocian" tablets near the village of Kültepe ($38^{\circ} 44' N$, $35^{\circ} 34' E$). These cuneiform tablets provide a foundation for study of the

pre-Hittite (*circa* 1940 BC to 1740 BC) network of Assyrian merchant colonies in Bronze Age Anatolia¹. Bilgic² has published the names of 119 towns active in this exchange, as evidenced by their citation on a tablet. The exact location of most of these sites is not known, and historical and archaeological investigations would be greatly facilitated if they could be found. We have used Bilgic's compilation to estimate the geographical position of several of the towns, in the sense of Bunge³, which is conditional on several assumptions. We invoke assumptions to enhance the analysis by applying the leverage obtained from geographical theory. We assume, for example, that mathematical models based on contemporary (largely western) data have a temporally and geographically invariant structure and can be applied to the Cappadocian situation. Our approach differs from several previous attempts^{1,2,4}, to which it should be considered complementary rather than competitive. For example, we do not require the existence of itineraries; the mere mention of two town names on the same tablet is taken to define a relation between these towns. We recognize that one of the simplest geographical relations is distance and attempt to convert the tabular data into geographical distances. If this is possible then it is an almost routine matter to convert these distances into relative positions. Thus it may be possible to specify the actual locations of the towns; that is, to predict their latitude and longitude. The simplest model asserts that places which are mentioned together frequently are probably closer together than are places which are not mentioned together frequently. This spatial decay is similar to the temporal decay of linguistic relations postulated in glottochronology⁵, or of isotopic changes used in radiocarbon dating⁶. Our procedure attempts to estimate spatial, rather than temporal, origins and can be considered the geographical equivalent to these methods. It also has comparable limitations. We know of no previous use of the procedure presented here.

On a purely random basis, one would expect the names of large towns to occur more frequently than the names of small towns. The total expectation is thus that the interaction between places depends on the size of the places and the separation between the places. This rather obvious result has been verified in a large number of societies and for many phenomena⁷. Specifically, we expect the interaction to increase as the places get bigger, and to decrease as they are farther apart. Many functions satisfy such a requirement. For social interaction the most common formulation is the so-called gravity model⁸

$$I_{ij} = k P_i P_j / d_{ij}^2$$

where I_{ij} is the interaction between places i and j ; k is a constant, depending on the phenomena; P_i is the population of i ; P_j is the population of j ; and d_{ij} is the distance between places i and j .

Distance may be in hours, dollars, or kilometres; populations may be in income, numbers of people, numbers of telephones and so on; and the interaction may be in numbers of letters exchanged, number of marriages, similarity of artefacts or cultural traits and so on. The evidence that it "works" has been assembled for more than 20 yr; why such an equation should work is sometimes interpreted as a metaphysical question and continues to be debated⁹. Here, we assume that this model might hold for the number of joint occurrences of place names in merchants' letters. We know of no specific data on this topic, although a modern empirical test seems feasible. The gravity model can of course be inverted to solve for the distance d_{ij} , as can several alternate interaction models¹⁰. For our purpose this is essential. The step from distances to coordinates can be solved by iterative least squares trilateration techniques¹¹. These have recently been generalized in psychology under the heading of multidimensional scaling¹². For the computation we have used a non-metric computer program made available by Lingoes¹³. This procedure attempts to preserve only the rank order of the distances, not their absolute

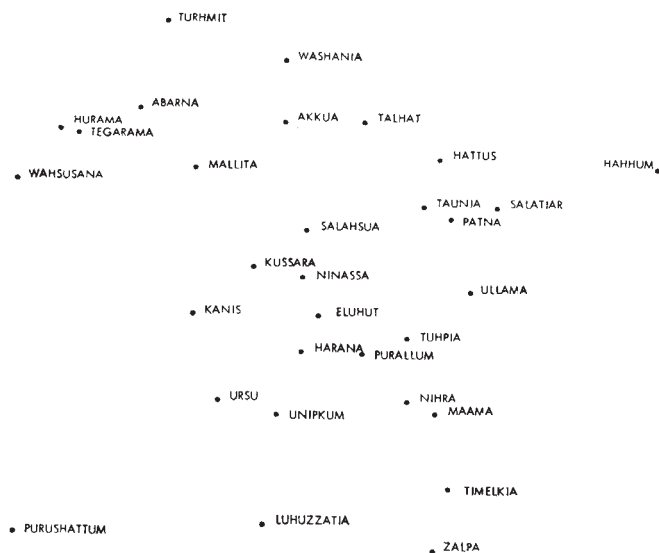


Fig. 1 Predicted location of the more important of the pre-Hittite towns. Based on interaction among sixty-two towns as evidenced by citation on cuneiform tablets. See text for details.

ratios, and thus partitions spatial interaction models into monotonic equivalence classes. Shepard¹⁴ has emphasized the value of the weaker ordinal assumptions and that the form of the model can be determined after the analysis. This is because the geometric constraints override inconsistencies in the data and minor distinctions between alternate models. The specific equation used for interaction is thus of no great significance.

Bilgic's table gives, for each of 119 towns, the tablets on which the name of that town occurs (a total of 819 tablets is thus referenced). We have taken the number of occurrences of a town name to be proportional to the population of that town. The table can be inverted to list place names by tablet and this is quickly converted to a frequency count. Sixty-five tablets are eliminated from the ensuing analysis since they lead to dead ends, in the sense that they contain the sole reference to a particular town. There remain sixty-two towns occurring on at least two of 754 tablets. These results are most conveniently arrayed in a table of sixty-two rows (one for each town), and sixty-two columns, with the entry at the intersection of any row and column made equal to the number of times that the names of the particular towns occur together on a tablet. This table of joint-mention frequencies is symmetrical so that only the lower half need be completed. The site near Kültepe has been identified as the Assyrian karum Kanis and most of the tablets can thus be considered to bear the implicit imprimatur "found at Kanis". Thirteen of the tablets (OIP XXVII 1-53) were excavated at Alishar (35° 35' N, 35° 15' E), commonly identified as Akkua¹⁵, a small number of Cappadocian tablets have also been uncovered at Bogazköy (40° 02' N, 34° 37' E), now known to have been the Hittite capital Hattus. Out of the possible $n(n-1)/2 = 1,891$ connexions, only 187 actually occur if we ignore the implicit imprimatur (which would add forty-four new connexions) and the Alishar tablets (adding nine more connexions if Akkua is assumed to be implicit on these tablets). This is approximately 10% of all possible connexions, and averages three connexions per town. The actual numbers of connexions ranges from a low of two to a high of twenty-three. The frequency ranges from a low of one joint mention to a high of twelve joint mentions. Some towns are mentioned only once, Purushattum 101 times (seventy-two times in isolation and on twenty-nine tablets with at least one other place name). Our entire analysis is based on Bilgic's work, which is the only currently published complete tabulation. Obvious minor typographical errors have been corrected but Bilgic may have confused regional

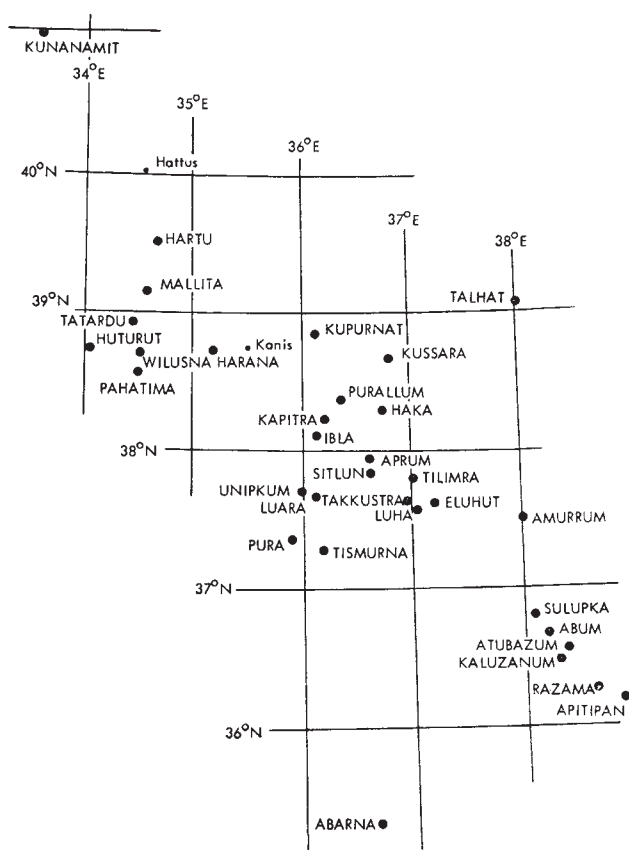


Fig. 2 Predicted location of thirty-three pre-Hittite towns, contingent on the assumption that the towns shown on Orlin's map are correctly located. See text for details.

names with town names, or mistranslated a personal name as a town name, or confused the names of two similar towns, and so on. The most obvious improvement to our analysis would be to use a more extensive collection of data, such as a tabulation prepared from all of the 17,000 Cappadocian tablets excavated so far. Such compilations are in preparation¹⁶ but were not available to us.

Our experiment resulted in the configuration shown in Fig. 1, which is based on all the joint mentions and the estimated populations, without constraints to fix the positions of any locations. The fit of this figure to the available data is high (>80%) as is usual for the gravity model, but this is mostly a measure of the internal consistency of the data. The more critical test is to compare our results with known sites. Any solution results in relative coordinates and at least two points must be known in absolute coordinates to determine the scale, and a third point to determine the absolute orientation. For statistical stability many positions should be known in advance. In this case there are sixty-two points to be located and only Kanis and Hattus (perhaps also Akkua) can be considered known, although reasonable speculations are available concerning the locations of several other sites. In a formal sense our results would allow calculation of a latitude and longitude coordinate for each town, but such precision does not seem warranted until further data become available for analysis.

We can of course compare our results with other published estimates, most of which are based on a few itineraries. In many cases the interpretations differ and the authors do not agree with each other. Locations are rarely precise, often indicating only the general area. Geographical maps require a more precise specification and we have been able to compare our results with the map given by Orlin¹⁷. Twenty-nine towns are common to the two studies; the correlation coefficient comparing the twenty-nine locations as given by these

two studies is essentially zero. This is disappointing. Both are speculations, of course, one based on a few specific details, the other on 754 tablets indicating general interaction. Our statistical procedure would be more convincing if an extensive network of sites were known exactly and only one or two additional locations were to be found. Orlin's data allow such an estimate to be made, for if it is assumed that his map is correct then this can be incorporated as a weighted constraint in the computer program. On this basis thirty-three locations have been computed from the interaction data and the twenty-nine fixed positions (Fig. 2). We thus obtain coordinates with a precision which is contingent on empirical data, on geographical location theory, and on previous Assyrian scholarship. Five typical results are:

Harana	38° 44' N ± 22'	35° 04' E ± 32'
Huturut	38° 46' N ± 11'	34° 03' E ± 31'
Kussara	38° 40' N ± 21'	36° 49' E ± 35'
Pahatima	38° 33' N ± 04'	34° 31' E ± 10'
Tilimra	37° 49' N ± 70'	37° 01' E ± 49'

The probable errors are obtained as an automatic byproduct of our procedure and can be interpreted in the usual manner. The average of roughly 50 km may seem too large for the field archaeologist, especially in an area as rich in mounds as central Turkey, but this does not invalidate the technique. As a tool for investigation it has specific data requirements and thus provides a focus for investigation. As in the dating of sites a convergence of evidence is more convincing than a single indicator. Particular sites may deviate from the model but this is then an important method of drawing attention to anomalous situations worthy of more detailed investigation.

W. TOBLER
S. WINEBURG

Department of Geography,
University of Michigan,
Ann Arbor, Michigan 48104

Received October 8, 1970; revised April 13, 1971.

- Garelli, P., *Les Assyriens en Cappadoce* (Maisonneuve, Paris, 1963).
- Bilgic, E., *Arch. Orientforsch.*, **50**, 1 (1951).
- Bunge, W., *Theoret. Geog.* (Gleerup, Lund, 1966).
- Garstang, J., and Gurney, O., *The Geography of the Hittite Empire* (British Institute of Archaeology, Ankara, 1959).
- Gudschinsky, S., *Word*, **12**, 175 (1956).
- Libby, W., *Science*, **159**, 621 (1961).
- Olsson, G., *Distance and Human Interaction* (Regional Science Association, Philadelphia, 1965).
- Isard, W., et al., *Methods of Regional Analysis* (Wiley, New York, 1960).
- Niedercorn, J., and Bechdolt, B., *J. Reg. Sci. Assoc.*, **9**, 273 (1969).
- Tobler, W., Detwyler, T., and Mielke, H., *Bioscience*, **20**, 537 (1970).
- Wolf, P., *Surveying and Mapping*, **29**, 635 (1969).
- Guttman, L., *Psychometrika*, **33**, 469 (1968).
- Lingoes, J., and Roskam, E., *Multivariate Behavioral Research*, **5**, 183 (1970).
- Shepard, R., *J. Math. Psychol.*, **2**, 287 (1966).
- Gelb, I., *Oriental Institute Publications*, 27 (University of Chicago Press, 1935).
- Gardin, J., in *The Use of Computers in Anthropology* (edit. by Hymes, D.) (Moulton, The Hague, 1965).
- Orlin, L., *Assyrian Colonies in Cappadocia* (Moulton, The Hague, 1970).

Surface Wave Momentum and Energy Velocities

REYNOLDS¹ defined the energy velocity associated with a simple periodic wave train on the surface of deep water as the phase velocity multiplied by the ratio of the energy transmitted across a fixed vertical plane during one wave period to the energy of the waves per wavelength. In other words,

the energy velocity is the velocity of a moving vertical plane across which, on average, no energy is transmitted. For waves of small amplitude this velocity is half the phase speed (see also ref. 2). Starr and Platzman³ extended the Reynolds concept to the case of waves of finite height, without mathematical approximation or further physical assumptions. The ratio already mentioned of energy transmitted, E_t , to the energy per wavelength, E , is now $E_t/E = \frac{1}{2} + 5\epsilon/2E$, where ϵ is the excess of kinetic over potential energy per wavelength, which vanishes for waves of small amplitude⁴. The phase speed for finite waves, multiplied by the ratio, gives the energy velocity which, for the highest waves, is 11/17 (or about 0.647) of this speed (see also *Handbuch der Physik*⁵ for a review of this and background material).

The concept of Reynolds, in the same circumstances as those stated, can be generalized to apply not only to the energy transport, but in a similar manner also to momentum transport, in the case of finite waves. Both the momentum and its transport are defined as being in the direction of wave propagation. I have shown⁶, again by exact methods in the sense already stated, that the ratio of the momentum transmitted, M_t , to the momentum per wavelength M , is $M_t/M = \frac{1}{2} + 3\epsilon/2e$, where e is the kinetic energy per wavelength, and ϵ is as before. The phase speed for finite waves, multiplied by the ratio, now gives the momentum velocity which, for the highest waves, is 2/3 (or about 0.667) of this speed.

The difference between the momentum and energy velocities is rather small for the highest waves, and for less steep waves it becomes extremely small. But, its presence suggests in a general way, that long but finite wave groups cannot move for extended time periods without secular changes in their physical energy spectrum in the sense of Rossby⁷ (see also *Handbuch der Physik*⁵) who demonstrated the invariance of such a spectrum for the small amplitude theory of surface waves in deep water.

V. P. STARR

Department of Meteorology,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Received March 8, 1971.

¹ Reynolds, O., *Nature*, **16**, 343 (1877).

² Lamb, H., *Hydrodynamics* (Cambridge University Press, 1932).

³ Starr, V. P., and Platzman, G. W., *J. Mar. Res.*, **7**, 229 (1948).

⁴ Platzman, G. W., *J. Mar. Res.*, **6**, 194 (1947).

⁵ Wehausen, J. V., and Laitone, E. V., *Handbuch Phys.*, **9**, 446 (1960).

⁶ Starr, V. P., *Geof. Pura e Appl.*, **48**, 109 (1961).

⁷ Rossby, C.-G., *J. Mar. Res.*, **6**, 93 (1947).

BIOLOGICAL SCIENCES

Prebiotic Formation of Cytidine Nucleotides

IN contrast to the plausible explanations for proteinoid formation¹, there seems to be no satisfactory concept for the genesis of polynucleotide templates in the presumed conditions of the primitive Earth. Only parts of the problem, such as the origin of the bases^{2,3} and carbohydrates⁴ and the synthesis of short oligonucleotides^{5,6}, have been subjected to informative experiment. A working hypothesis of the past decade took for granted the availability of common nucleosides, but only in 1970 was a plausible, though inefficient, route to any natural nucleosides proposed⁷. Some recent evidence suggests, however, that natural nucleosides need not be direct precursors of the corresponding nucleotides and polynucleotides.

Sanchez and Orgel⁷ have developed a method in which $0^2:2'$ -cyclocytidine and $0^2:2'$ cyclouridine, among others,

are formed in high yield in potentially prebiotic conditions. Various cyclonucleoside phosphates and their polymers can be obtained by rearrangements of the mono and polyribonucleotides of the pyrimidine bases which have been studied in several laboratories⁸⁻¹². The results of these studies suggest that cyclonucleotides and arabinonucleotides are more stable ribonucleotides in conditions of thermal and chemical activation, and this could have influenced the chronological order of appearance, or predominance, of the corresponding carbohydrate moieties.

Since a reasonably good case can be made for the prebiotic availability of cyclonucleosides, it is important to inquire whether ribonucleotides could be derived from them. Two such cyclo→ribo conversions, conducted in ungeological conditions, have been mentioned briefly^{11,13}. Here we report that $0^2:2'$ -cyclocytidine 3'-phosphate⁹ (I) can be converted in high yield to cytidine 2':3'-cyclic phosphate; we suggest that this reaction could have been a route to the prebiological formation of cytidine derivatives, including oligocytidylates.

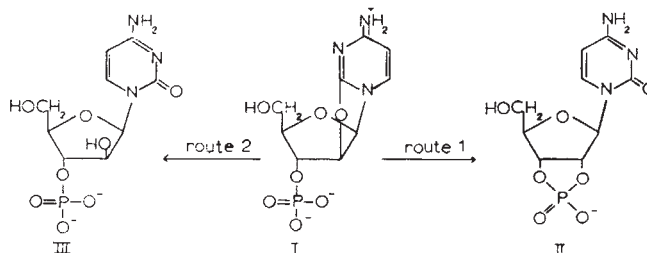


Fig. 1

The chemistry of this reaction involves the formation of the cyclic phosphodiester by an intramolecular nucleophilic displacement involving the phosphomonoester and the 2'-cyclo-nucleoside moieties (Fig. 1, route 1). This "cyclonucleoside method" of phosphorylation¹⁴ has been performed so far only on the 5' and 3'-cyclonucleosides in intermolecular reactions. For optimum reactivity in the displacement, the phosphate must be in the dianionic form; in acidic solutions with a pH less than its pK (5.7), compound I is quite stable. The zwitterionic free acid is also stable in solid state up to 140°C, the temperature at which slow decomposition begins. Above pH 8 the hydrolysis of the cyclonucleoside takes place rapidly by general base catalysis, shown in Fig. 1 as route 2, and the only product is aracytidine 3'-phosphate (III). The two routes are concomitant at pH 7, and their ratio depends on the nature of the anions. One of the most outstanding observations summarized in Table 1 is the great difference in the catalysis of the hydrolysis by the bicarbonate and chloride ions. This rate difference may be indicative of an intramolecular catalysis by the divalent species. The reactivity of compound I in water at pH 7 shows the profound influence of the ionic strength on the stabilization of the ion pair. While no reaction took place in 1 M chloride solution at 37°C for 2 days because of an efficient shielding of the phosphate and immonium ions, a quantitative conversion of I to a mixture of II (67%) and III (33%) was observed in 0.05 M chloride.

The high ratio of ribo→arabino that can be easily achieved in aqueous solution is a significant finding in the context of evolution. The hydrolysis can be restricted and route 1 enhanced when organic solvents are used, or, even more effectively, when the triethylammonium salt of I is heated in the solid state. Prolonged heating in the absence of moisture led to the formation of a small amount of polymeric material at the expense of II.

Cytidine 2':3'-cyclic phosphate was characterized by complete hydrolysis with RNAase I to cytidine 3'-phosphate and subsequent alkaline phosphatase hydrolysis. The nucleosidic products obtained from II and III were cytidine and 1-β-D-arabinofuranosylcytosine, identified on the basis of their identical mobility with the authentic samples in paper chromatography.

grams and by electrophoresis in borate buffer. Ultraviolet and optical rotary dispersion spectra were also identical.

To trace the origin of the polymeric material, the triethylammonium salt of cytidine 2':3'-cyclic phosphate was heated in solid state at 138° C for 48 h. This was done because Škoda *et al.*⁶ have shown a similar polymerization of uridine 2':3'-cyclic phosphate. The analysis of the product on 'Sephadex G-25' revealed the presence of oligomers up to hexamer in addition to some 50% unreacted starting material. The dinucleotide and trinucleotide fractions were quantitatively degraded by alkali, and treatment of the monomers with alkaline phosphatase produced only cytidine. It is interesting that the dimer contained approximately equal amounts of the two isomers, while the trinucleotide had slightly more (66%) of the unnatural 2'→5' linkages, determined by the action of pancreatic ribonuclease. Apparently, this simple thermal polymerization cannot produce appreciable quantities of all-natural polycytidylates.

Because the process we have described is simple, one may infer that it could have been operational in prebiological times. The prebiological relevance of this route, however, depends on the likelihood of the formation of 0²:2'-cyclocytidine 3'-phosphate from the corresponding nucleoside. On the basis of current knowledge of chemical phosphorylation, we assume that the prebiotic agents, whatever they were, could not have possessed an exclusive stereospecificity towards the primary hydroxyl group; some secondary phosphate esters could have been formed in addition to the predominant 5'-monophosphate and 3',5'-diphosphate (our preliminary experiments tend to support this contention). It is important to note that 0²:2'-cyclocytidine 3',5'-diphosphate could undergo a similar rearrangement to 5'-phosphoryl cytidine 2':3'-cyclic phosphate which also could initiate the chain growth, or become a source of cytidine.

The question of whether or not 0²:2'-cyclocytidine 3'-phosphate and cytidine 2':3'-cyclic phosphate could have been sufficiently enriched in primordial conditions remains a disputable point, although there are considerable differences in the solubilities of the zwitterionic, monoanionic and dianionic nucleotides. This uncertainty is compensated for by the expected absence of interference of the 5'-nucleotide with the polymerization of cytidine 2':3'-cyclic phosphate. The chain growth is effected by transesterification involving primary hydroxyl groups only; the transition state with a secondary alcohol would be difficult to form. The same transesterification process is known to be facilitated by enzyme catalysis¹⁵ in which the natural internucleotide linkage is formed by a specific cleavage of the P—O(2') bond. Orgel has considered

Table 1 Reactivity of 0²:2'-Cyclocytidine 3'-Phosphate (I), as the Triethylammonium or Tri-n-octylammonium Salt

	Time h	Temp. °C	Unreacted %	Ratio ribo : arabino
Solid state	15	116	0	5.6
0.05 M Tris-Cl	24	37	8	
pH 7.0	48	37	0	2.0
1 M NaCl	48	37	99	—
0.05 M NaHCO ₃				
pH 7.0	24	37	0	0.02
Dimethylformamide	15	80	0	4.0

The concentrations of the triethylammonium and tri-n-octylammonium salts were 0.01–0.05 M. Before the determination of the ribo : arabino ratio, the complete disappearance of the starting material was checked on electrophoretograms developed in 0.03 M sodium phosphate (pH 6.0) at 50 V/cm. Relative mobilities: III, 1.0; II, 0.7; I, 0.5. The reaction products were first hydrolysed with 0.3 M KOH for 17 h at 37° C, then after neutralization with 'Dowex 50(H⁺)' and filtration, the sample was made 0.05 M in Tris-chloride (pH 8.5) and incubated with alkaline phosphatase (Worthington, BAPF). The nucleosides were separated in 1-butanol-water 84:16, or 2-propanol-conc. ammonia-water 7:1:2. The ribo : arabino ratio was determined spectroscopically, after elution with water, at 272 nm.

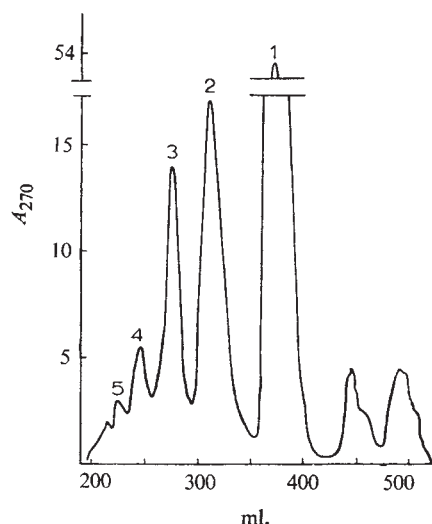


Fig. 2 Gel permeation chromatography of oligocytidylates. The polymerization of cytidine 2':3'-cyclic phosphate, triethylammonium salt, was carried out *in vacuo* at 138° C for 48 h, and the product was passed through a 'Sephadex G-25', superfine, column (100 × 2.5 cm) in 0.2 M triethylammonium bicarbonate at a rate of 9 ml/h. The chain length is indicated by the numbers over the peaks. The last of the unnumbered fractions contained cytosine, the other was not identified. The yields of the di, tri, tetra, and pentanucleotides were 16, 10, 4 and 2% respectively.

the feasibility of a non-enzyme catalyst performing a similar polymerization (personal communication), but the origin of the required nucleoside cyclic phosphates is not yet clear. The availability of suitable substrates, as we propose, seems to increase the probability that the transesterification route played a major role in the prebiotic evolution of polyribonucleotides of the pyrimidine bases.

Since the corresponding purine cyclonucleosides do not exist, the path we have described is not applicable to the formation of purine polynucleotides. This fact and the observation that only pyrimidine polynucleotides can serve as templates for chemical replication¹⁶ is an intriguing coincidence which deserves emphasis, if not another hypothesis.

We thank Dr L. E. Orgel for disclosing much of his work before publication, and Drs J. Oró and J. Žemlička for reading our manuscript. This research was supported by the National Institutes of Health, US Public Health Service.

CLAUDE M. TAPIERO
JOSEPH NAGYVARY

Texas A and M University,
Department of Biochemistry and Biophysics,
College Station, Texas 77843

Received July 6, 1970.

- 1 Fox, S. W., *Origins of Prebiological Systems and of their Molecular Matrices*, 361 (Academic Press, New York, 1964).
- 2 Oró, J., *Biochem. Biophys. Res. Commun.*, **2**, 407 (1960).
- 3 Ferris, J. P., Sanchez, R. A., and Orgel, L. E., *J. Mol. Biol.*, **30**, 223 (1967).
- 4 Mariani, E., and Torracca, G., *Intern. Sugar J.*, **55**, 309 (1953).
- 5 Ponnampuram, C., and Mack, R., *Science*, **148**, 1221 (1965).
- 6 Škoda, J., Morávek, J., and Kopecký, J., *Sixth FEBS Meeting, Abstr.*, 433 (1969).
- 7 Sanchez, R. E., and Orgel, L. E., *J. Mol. Biol.*, **47**, 531 (1970).
- 8 Walwick, E. R., Roberts, W. K., and Dekker, C. A., *Proc. Chem. Soc.*, 84 (1959).
- 9 Nagyvary, J., *J. Amer. Chem. Soc.*, **91**, 5409 (1969).
- 10 Schramm, G., and Ulmer-Schurnbrand, I., *Biochim. Biophys. Acta*, **145**, 7 (1967).
- 11 Nagyvary, J., and Provenzale, R. G., *Biochemistry*, **8**, 4769 (1969).
- 12 Provenzale, R. G., and Nagyvary, J., *Biochemistry*, **9**, 1744 (1970).
- 13 Walwick, E. R., thesis, Univ. California, Berkeley (1958).
- 14 Mizuno, Y., and Sasaki, T., *J. Amer. Chem. Soc.*, **88**, 863 (1966).
- 15 Bernfield, M. R., and Rottman, F. M., *J. Biol. Chem.*, **242**, 4134 (1967).
- 16 Orgel, L. E., *J. Mol. Biol.*, **38**, 381 (1968).

Flux Ratios and Isotope Interaction in an Ion Exchange Membrane

IN spite of the widespread use of radioactive tracers in the study of permeation of biological tissues, it is well known that the permeability coefficient derived from tracer exchange may differ appreciably from that derived from net flow¹⁻⁴, as, for example, for water flow in the frog skin and toad bladder¹⁻³ and sugar transport in red blood cells⁴. There is a similar ambiguity in the use of the "flux ratio" to evaluate the forces promoting transport. For simple passive flow across a membrane the original formulation^{2,5,6} for the ratio of "unidirectional fluxes" was given as

$$f = \exp(X/RT) \quad (1)$$

where X is the negative electrochemical potential difference of the test species, and R and T are the gas constant and absolute temperature, respectively. Deviations from this formula (that is an "abnormality" of the flux ratio) were considered to indicate additional forces such as solvent drag or active transport. Although the flux ratio equation has been useful in characterizing the forces which influence net transport, important exceptions have been noted^{1,2,7,8}. A clear example is the potassium flow in the poisoned squid axon, in which the flux ratio is markedly abnormal in spite of the apparent absence of either solvent drag or active transport⁷.

Although these difficulties have been appreciated and explained in mechanistic terms, there has been no unifying treatment. To provide a general formulation Kedem and Essig⁹ examined the theory of isotope flows in terms of non-equilibrium thermodynamics. In their view, both the above anomalies can be attributed quite generally to coupling between the flows of the abundant and tracer species ("isotope interaction"). The formulation is particularly simple if net flow of the test species is the result only of its electrochemical potential difference, so that the phenomenological resistance coefficient for net flow is given by

$$R = X/J \quad (2)$$

In the presence of isotope interaction, R is unequal to the

exchange resistance R^x , where

$$R^x = (-RT \Delta\rho/J^x)_{J=0} \quad (3)$$

(Here J and J^x are the net flow and tracer flow, respectively, and $\Delta\rho$ is the difference in specific activity across the membrane.) Furthermore, with isotope interaction the flux ratio is abnormal even in the absence of solvent drag and active transport, being given by

$$f = \exp[(R^x/R)(X/RT)] \quad (4)$$

In the absence of isotope interaction, $R^x = R$, and equation (4) reduces to equation (1).

Although the assumptions for the above formulation are broadly valid, suggesting its general usefulness, it has not been tested experimentally. Gottlieb and Sollner's observations (that collodion membranes activated with certain quaternary ammonium polyelectrolytes show tracer exchange rates greater than predicted from their electrical resistance)¹⁰ provided an opportunity. We demonstrate here that the flux ratio in these membranes is "abnormal", according to equation (1), and that its value is predicted quantitatively by the modified expression (equation (4)).

Membranes were prepared by adsorption of polyvinylbenzyl-trimethyl ammonium chloride (PVBT, Dow) on a collodion matrix, as described previously^{10,11}. Their usefulness was increased by their near-ideal permselectivity, which permitted the evaluation of net anion flow from electrical current. The membranes were exposed at each surface to 0.1 M KCl. R was evaluated from the electrical resistance, and R^x from the self-diffusion of ³⁶Cl (Table 1). In several membranes it was possible to reproduce the effect noted by Gottlieb and Sollner, with values of R^x/R as low as 0.42.

We were able to test the modified flux ratio equation using membranes with unequal values of R^x and R . Unidirectional fluxes were evaluated from two series of measurements of tracer flow, one with and one against the electrical potential gradient. The flux ratio f , abnormal according to equation (1), was closely predicted by equation (4). We also found, unexpectedly, that not all PVBT-collodion membranes had a significant difference between R^x and R . (Gottlieb has also

Table 1 Theoretical and Observed Flux Ratios in PVBT-Collodion Membranes

Membrane	$\Delta\psi$ (mV)	R^x (10 ⁷ kilo-calories cm ² s mol ⁻²)	R^x/R	$\exp(X/RT)$	f	$\exp\left(\frac{R^x X}{R RT}\right)$
D	10	14.1	0.71	1.48	1.34	1.32
D	25	14.1	0.69	2.65	2.05	1.96
A	25	14.8	0.44	2.65	1.56	1.53
A	50	14.8	0.42	7.02	2.33	2.26
K	50	35.9	0.57	7.02	3.17	3.01
H	50	73.0	0.60	7.02	3.02	3.22
H	75	64.9	0.61	18.59	4.96	5.89
H	75	42.0	0.53	18.59	4.48	4.69
F	10	18.8	1.04	1.48	1.50	1.50
F	10	18.1	0.97	1.48	1.51	1.47
E	25	113	1.00	2.65	2.80	2.68
E	50	108	1.07	7.02	7.88	8.01

$\exp(X/RT)$ is the flux ratio predicted by equation (1), f is the observed flux ratio, and $\exp\left(\frac{R^x X}{R RT}\right)$ is the flux ratio predicted by equation (4). These quantities were evaluated as follows: membranes in lucite chambers were exposed at each surface to 0.1 M KCl. The "outer" solution (left side) contained high specific activity K³⁶Cl. The electrical potential difference ($\Delta\psi = \psi^{\text{in}} - \psi^{\text{out}}$) was measured by means of calomel half cells and 3 M KCl-agar bridges apposed to the membranes. Ag-AgCl electrodes and a voltage clamp were used to set $\Delta\psi$ as desired. The d.c. electrical resistance $\mathcal{R}$ of 1 cm² of membrane was determined from Ohm's law, transiently setting the potential at ± 10 mV. Because the chloride transport number was > 0.99 , the phenomenological resistance coefficient was taken as $R = F^2 \mathcal{R}$, where F is the Faraday. Tracer fluxes J^x and "unidirectional fluxes" $J^x/\Delta\rho$ were determined in three series of experiments, with $\Delta\psi$ zero, positive, and negative, respectively. After each series (three or four 10-15 min periods) the cold solutions were replaced. At zero potential difference, $J = 0$, thus R^x could be calculated from equation (3). The flux ratio was calculated from $f = \text{influx/outflux}$, taking the mean value of $(J^x/\Delta\rho)_{\Delta\psi+}$ as the influx, and that of $(J^x/\Delta\rho)_{\Delta\psi-}$ as the outflux. This means of determining the outflux is experimentally simpler than reversal of the hot and cold sides. The use of an equation ((28) of ref. 9), which incorporates the effect of backflux precisely, did not alter f significantly.

noted this variability in PVBT-collodion membranes (personal communication), but the factors accounting for the variability of R^*/R are so far undefined.) In these membranes, which served as useful controls, the flux ratio was "normal". (It is believed that electro-osmotic flow is insignificant in the membranes we used¹⁰. If appreciable, the values of R^*/R would be smaller than reported, but the fundamental formula (equation (29) of ref. 9) is still valid.)

Our results substantiate the thermodynamic formulation presented previously, and therefore support the view that anomalous tracer permeability coefficients and flux ratios are due to coupling of isotope flows (in this case negative coupling)⁹. In biological membranes, flux ratios between exp (X/RT) and unity as observed here are usually attributed to exchange diffusion by means of mobile carriers². Other mechanisms are plausible, however, on theoretical grounds¹²⁻¹⁴. It is unlikely that carriers can traverse the synthetic membranes studied here, which suggests that such carriers may not be the mechanism of abnormal flux ratios in biological membranes.

We thank Drs M. Gottlieb and K. Sollner for samples and advice and D. Nadel for technical assistance. This work was supported by grants from the US Public Health Service and the Office of Saline Water. R. C. S. is a fellow of the Massachusetts Heart Association, J. H. L. is a Charlton Fund fellow and A. E. a USPHS development awardee.

R. C. deSOUSA
J. H. LI
A. ESSIG

Renal Unit,
New England Medical Center Hospitals
and Tufts University School of Medicine,
Boston, Massachusetts 02111

Received November 9, 1970.

- ¹ Koefoed-Johnsen, V., and Ussing, H. H., *Acta Physiol. Scand.*, **28**, 60 (1953).
- ² Ussing, H. H., in *Alkali Metal Ions in Biology* (Springer, Berlin, 1960).
- ³ Hays, R. M., and Leaf, A., *J. Gen. Physiol.*, **45**, 905 (1962).
- ⁴ LeFevre, P. G., and McGinniss, G. F., *J. Gen. Physiol.*, **44**, 87 (1960).
- ⁵ Ussing, H. H., *Acta Physiol. Scand.*, **19**, 43 (1949).
- ⁶ Teorell, T., *Arch. Sci. Physiol.*, **3**, 205 (1949).
- ⁷ Hodgkin, A. L., and Keynes, R. D., *J. Physiol.*, **128**, 51 (1955).
- ⁸ Levi, H., and Ussing, H. H., *Acta Physiol. Scand.*, **16**, 232 (1948).
- ⁹ Kedem, O., and Essig, A., *J. Gen. Physiol.*, **48**, 1947 (1965).
- ¹⁰ Gottlieb, M. H., and Sollner, K., *Biophys. J.*, **8**, 515 (1968).
- ¹¹ Gottlieb, M. H., Neihoff, R., and Sollner, K., *J. Phys. Chem.*, **61**, 154 (1957).
- ¹² Essig, A., Kedem, O., and Hill, T. L., *J. Theoret. Biol.*, **13**, 72 (1966).
- ¹³ Shean, G. M., and Sollner, K., *Ann. NY Acad. Sci.*, **137**, 759 (1966).
- ¹⁴ Snell, F. M., Shulman, S., Spencer, R. P., and Moos, C., *Biophysical Principles of Structure and Function*, 320 (Addison-Wesley, London, 1965).

Serum Proteins after a Liver Transplant

PROTEIN concentrations after liver transplantation are of interest because most plasma proteins, other than the immunoglobulins, are made wholly or partly in the liver. The concentrations of eighteen immunologically distinct proteins have been measured by a quantitative immunoelectrophoresis technique¹ in a single subject before and for 4.5 months after receiving an orthotopic liver graft.

The recipient was a 41 year old man with a primary hepatoma and the donor liver came from a 13 year old boy, who died from head injuries. After the operation, carried out by Professor R. Y. Calne, the patient made a good recovery and was discharged on the seventeenth day. During the second week he showed some evidence of rejection, but after a temporary increase in immunosuppressive therapy he improved and

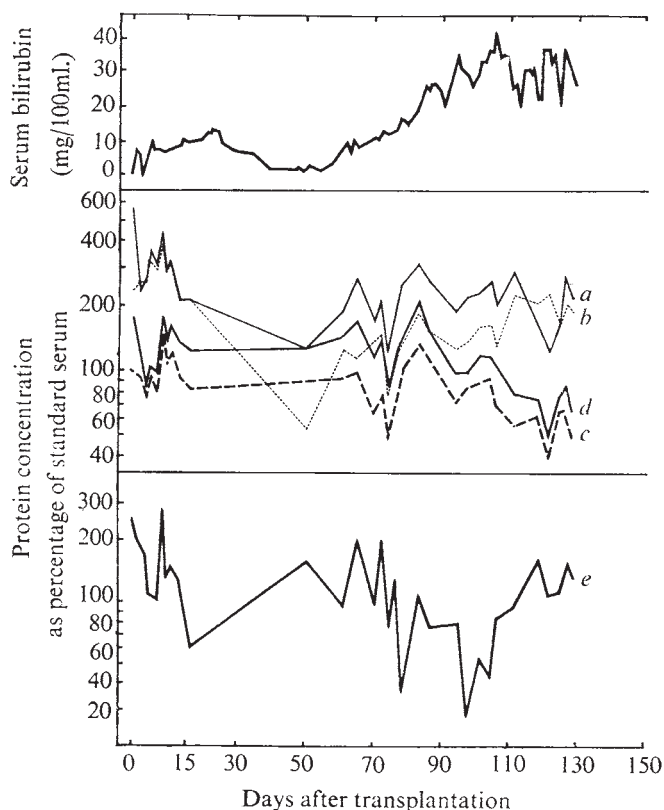


Fig. 1 Serial serum concentrations of haptoglobin (a), α_1 antitrypsin (b), albumin (c), transferrin (d) and β_1 A-C globulin (e) expressed as a percentage of a standard serum. Serum bilirubin concentrations are also shown.

was able to return to work in the sixth week. Unfortunately, he later developed progressive jaundice which was attributed to chronic rejection and died at 4.5 months from fungal meningitis².

The changes in serum protein concentrations observed led to the definition of two principal groups of proteins. Group (A) included those proteins which stayed at a normal or increased concentration from the time of transplantation to death. These proteins included haptoglobin, α_1 antitrypsin, caeruloplasmin, group component, and α_1 acid glycoprotein (Fig. 1). The concentrations of all these proteins were increased pre-operatively, probably because of necrosis in the tumour, and the concentrations remained high after the transplant. These proteins are often called acute-phase proteins, and Werner and Cohnen³ showed that their concentrations increased after surgery, but returned to pre-operative levels within 18 days of uncomplicated surgical trauma. The persistence of increased concentrations in our patient beyond this time was probably a reaction to the early rejection episode. At the fifty-sixth day, the concentrations of these and the other sixteen plasma proteins measured were normal, but during the subsequent prolonged phase of chronic rejection, the acute-phase protein concentrations were again maintained above normal.

Group (B) proteins included those that decreased gradually in concentration after the transplant, particularly after the eightieth day. They included albumin, transferrin and haemopexin. The α_1 lipoprotein concentrations also decreased throughout the study but at a greater rate than the other proteins mentioned. Because there was no reason for increased catabolism, the low concentrations of these proteins suggest either that there was no stimulus for synthesis or that the liver was unable to respond because of impaired parenchymal cell function. It is interesting therefore that at the same time the liver was able to maintain other proteins such as haptoglobin and α_1 antitrypsin at concentrations above normal. These changes may be a consistent feature of the acute-phase reaction^{3,4}.

The changes in two other proteins not included in our two groups are interesting. Thyroxine binding prealbumin behaved differently from the other proteins in that its concentration decreased initially, at the time of the marked increase in acute phase proteins, and again during the second increase in acute phase proteins after the eightieth day. Similar changes have been observed after simple surgery and virus infections⁴. The concentration of β_1 A-C globulin (the third component of complement C'3), which is probably synthesized solely in the liver⁵, decreased significantly in the first 17 days at the time of the acute rejection episode (Fig. 1). At the beginning of the period of chronic rejection, the concentration was also greatly depressed but later, when infection may have been an important stimulus to synthesis, it increased to normal. Hypercatabolism of C'3, sometimes with a decline in serum concentration, has been shown during episodes of renal graft rejection⁶ and this seems a more likely explanation than poor hepatic synthesis for the changes observed in our patient during rejection of the liver graft, as increased concentrations were found later in the clinical course when hepatic function was considerably impaired.

IAIN M. MURRAY-LYON
ROGER WILLIAMS

King's College Hospital,
London SE5

T. FREEMAN*
H. G. M. CLARKE

Clinical Research Centre,
Northwick Park Hospital,
London

Received December 3, 1970; revised January 13, 1971.

*Dr Freeman died on November 11, 1970.

- ¹ Clarke, H. G. M., and Freeman, T., *Clin. Sci.*, **35**, 403 (1968).
- ² Williams, R., et al., *Brit. Med. J.*, **3**, 12 (1969).
- ³ Werner, M., and Cohnen, G., *Clin. Sci.*, **36**, 173 (1969).
- ⁴ Clarke, H. G. M., and Freeman, T., *Clin. Sci.* (in the press, 1971).
- ⁵ Alper, C. A., Johnson, A. M., Birtch, A. G., and Moore, F. D., *Science*, **163**, 286 (1969).
- ⁶ Carpenter, C. B., Ruddy, S., Shehadeh, I. H., Muller-Eberhard, H. J., Merrill, J. P., and Austen, K. F., *J. Clin. Invest.*, **48**, 1495 (1969).

Induction of Cellular DNA Synthesis in Human Leucocytes by Epstein-Barr Virus

THE discovery of Epstein and Barr¹ of an antigenically and biologically unique herpes-like virus (EBV) in cultured cells derived from Burkitt's lymphoma aroused much speculation regarding the possible aetiological role of this agent in human lymphoproliferative diseases. Serological and clinical data provided strong evidence for the causal relationship between EBV and infectious mononucleosis². The case for the possible association of EBV with Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC) rests mainly on the observation that patients with these diseases have significantly higher EBV antibody titres than matched controls from the same region³. Similar findings, however, were also reported in patients with sarcoidosis⁴ and systemic lupus erythematosus⁵.

Perhaps more direct evidence for the intimate association of EBV with BL and NPC was provided by a recent report⁶ which claims the detection of viral DNA in tumour biopsies. This finding, however, does not eliminate the possibility that these tumour cells became secondarily infected with this virus, which is known to occur in lymphoid cell cultures derived from healthy persons^{7,8}. Thus, in the absence of more convincing evidence, the possible "passenger" role for EBV is indeed difficult to exclude.

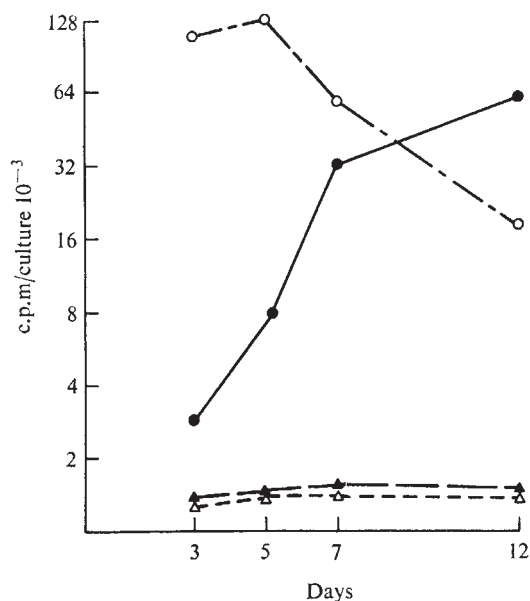


Fig. 1 Incorporation of ³H-thymidine (2 μ Ci/ml., specific activity 6.7 Ci/mmol) into leucocytes inoculated with EBV or *Candida albicans* extract. Cultures were incubated with the radiolabelled precursors for 16 h. Trichloroacetic acid precipitable material was dissolved in 'Hyamine' and counted in toluene and 'Liquifluor' in a scintillation counter. Uninoculated control (Δ); *Candida*-stimulated cells ($\circ$); and cells infected with EBV ($\bullet$). Note that stimulation is absent with inactivated EBV ($\blacktriangle$).

We have shown previously⁹ that leucocytes from adult human donors who had no previous EBV infection had a limited life-span *in vitro* and could be established in long term culture. On the other hand, infection of such cells with EBV resulted in lymphoblastoid transformation and unlimited growth potential of these cells in culture. We now present evidence that infection of human leucocytes with EBV induces cellular DNA synthesis.

Buffy coat cells were obtained from heparinized blood of healthy adults and were cultured in medium 1640 containing 20% autologous plasma and 80 μ g/ml. of neomycin sulphate. EBV was derived from culture fluids of AV cells⁹. The cell-free fluids were filtered through a 0.45 μ m 'Millipore' membrane and the virus was sedimented by centrifugation for 2 h at 20,000 r.p.m. The virus pellets were resuspended in 1/25 to 1/50 of the original volume in growth medium and stored at -70° C. These preparations were free of mycoplasma or other detectable contaminants. Virus infectivity, determined by titration in a virus-free lymphoid cell line⁹, ranged from 10^{-2} – 10^{-3} /0.2 ml. Pellets of 10 – 15×10^6 leucocytes were infected with 0.3 ml. EBV diluted 1:2 and incubated for 1 h at 37° C with periodic shaking. The cells were then resuspended in growth medium to a concentration of 1×10^6 /ml., distributed to 13×100 mm tubes and incubated at 37° C in the presence of 5% CO_2 . Virus infectivity was destroyed by heating at 56° C for 30 min or by ultraviolet irradiation. *Candida albicans* 10% allergenic extract (Hollister-Stier Laboratories) was diluted 1:10 and 0.1 ml. added to 10^6 leucocytes.

Representative results obtained in six experiments with leucocytes from donors without detectable complement-fixing antibodies to EBV are illustrated in Fig. 1. Leucocytes exposed to *Candida* exhibited the expected response of cells from sensitized donors to specific antigenic stimulation. Induction of DNA synthesis following EBV infection exhibited a stepwise increase during 12 days. Untreated leucocytes exhibited little or no uptake of tritiated thymidine. The EBV-induced stimulation of DNA synthesis could be completely inhibited by inactivation of virus infectivity. Similar treatment of the *Candida* extract had no effect on its stimulatory activity. Autoradiographs of EBV-infected cultures demonstrated that up to 20% of blastoid cells had incorporated ³H-thymidine between 7–9 days post-infection. The blastoid cells exhibited

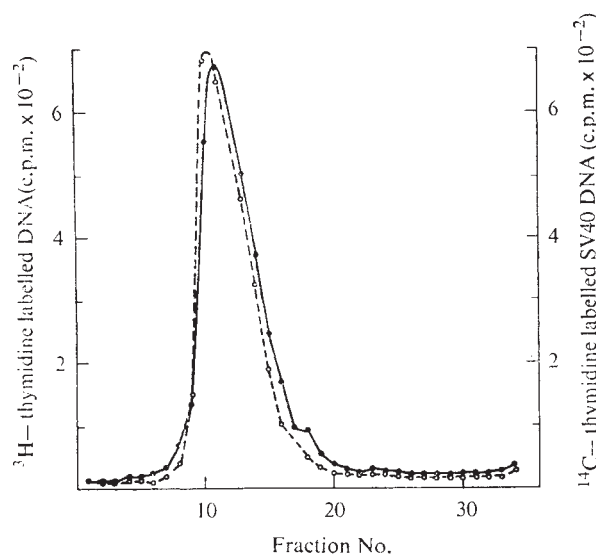


Fig. 2 Isopycnic bonding of ^3H -thymidine labelled EBV-stimulated DNA with ^{14}C -thymidine labelled SV40 DNA-I as a density marker. Fifty million EBV-stimulated leucocytes were digested for 2 h at 37°C in 5 ml. 0.05 M Tris-HCl, pH 8.2, 0.05 M EDTA, pH 8.0, in the presence of 1% sodium dodecyl sulphate and 2.5 mg pronase. Lysate (0.5 ml.) was mixed with the labelled SV40 DNA, diluted to 3.95 ml. with distilled water, and added to 4.85 ml. CsCl to yield an initial density of about 1.710 g/cm^3 . Tubes were filled with paraffin oil and centrifuged at 35,000 r.p.m. for 48 h in a Spinco No. 40 fixed angle rotor at 20°C . Tubes were sampled by puncture. Samples were precipitated with 10% trichloroacetic acid in the presence of 50 μg albumin, collected on membrane filters and examined for radioactivity by liquid scintillation spectrometry with differential ^3H and ^{14}C settings.

a marked increase in numbers and metabolic activity after 20–35 days, and became established lymphoid cell lines as described previously⁹. By contrast, both untreated control cells and antigen-stimulated cells failed to grow and gradually degenerated. Furthermore, DNA synthesis of leucocytes from donors possessing EBV antibodies was stimulated by either infectious or inactivated EBV preparations. These results indicate that the induction of DNA synthesis of leucocytes from EBV antibody negative donors was the result of active virus rather than a response to viral antigens alone. Furthermore, stimulation of lymphocytes with EBV antigens may offer a useful test to assess the degree of cellular immunity to EBV infection.

The ^3H -thymidine incorporated into acid precipitable components in cells stimulated by EBV preparations was predominantly non-viral. This was established by isopycnic centrifugation of lysates of 50×10^6 stimulated cells in CsCl as described by Flamm *et al.*¹⁰. The ^3H -thymidine labelled macromolecules banded isopycnically in nearly the same density region as ^{14}C -thymidine labelled SV40 virus DNA-I (supercoiled circles), which contains 42 mol % guanine and cytosine. Labelled EB virus DNA, if present in sufficient concentration, would have banded in denser regions of the CsCl gradient by virtue of its higher (66–68 mol %) guanine and cytosine content. Of interest is the lack of production of detectable concentrations of viral DNA, even though lysates from cells labelled for 18 h, 1 day, 5 days, and 9 days after infection were examined. These findings do not establish the labelled DNA to be representative of all of the DNA sequences of the host cell genome, even though the average mol % guanine and cytosine is similar to that expected for host DNA. The reassociation kinetics of the labelled DNA are being examined to determine if the polynucleotide sequences of the stimulated DNA are of a complexity similar to that of human DNA.

Activation of cellular DNA synthesis is unusual in cells infected by DNA viruses. Vaccinia or pseudorabies virus, for example, repress the synthesis of host DNA^{11,12}. The available evidence indicates that only oncogenic DNA viruses (polyoma,

SV40, adenovirus 12) can stimulate cellular DNA^{13–15} while oncogenic RNA viruses do not possess this function¹⁶. The similarity between cells transformed by oncogenic DNA viruses and EBV is further emphasized by the fact that the maintenance of the transformed state seems to depend on the continued presence of the EB viral genome. This is based on at least three lines of evidence: (a) EB viral DNA has been demonstrated in “virus-free” Raji cells derived from a Burkitt’s lymphoma¹⁷; (b) single-cell-derived clones of lymphoid cell lines free of detectable EBV gave rise to virus-containing progeny indicating the persistence of the viral genome in every cell of these cultures¹⁸; (c) soluble EBV-related complement-fixing antigen(s) are found in all established lymphoid cell lines regardless of the presence of detectable EBV¹⁹.

This study furnishes the first direct evidence of a function of EBV which is restricted only to oncogenic DNA viruses. These findings strongly suggest an aetiological role for this virus alone or in conjunction with other as yet unknown factors in certain lymphoproliferative disorders.

P. GERBER

*Division of Biologics Standards,
National Institutes of Health,
Bethesda,
Maryland 20014*

B. H. HOYER

*Carnegie Institution of Washington,
Department of Terrestrial Magnetism,
Washington, DC 20015*

Received February 26, 1971.

- ¹ Epstein, M. A., and Barr, Y. M., *Lancet*, i, 252 (1964).
- ² Henle, G., Henle, W., and Diehl, V., *Proc. US Nat. Acad. Sci.*, **59**, 94 (1968).
- ³ Levy, J. A., and Henle, G., *J. Bact.*, **92**, 275 (1966).
- ⁴ Hirshaut, Y., Glade, P., Octavio, L., Viera, B. D., Ainbender, E., Dvorak, B., and Siltzbach, L. E., *New Engl. J. Med.*, **283**, 502 (1970).
- ⁵ Evans, A. S., Rothfield, N. F., and Niederman, J. C., *Lancet*, i, 167 (1971).
- ⁶ Zur Hausen, H., Schulte-Holthausen, H., Klein, G., Henle, W., Henle, G., Clifford, P., and Santesson, L., *Nature*, **228**, 1056 (1970).
- ⁷ Gerber, P., and Birch, S. M., *Bact. Proc.*, 153 (1967).
- ⁸ Moore, G. E., Gerner, R. E., and Franklin, H. A., *J. Amer. Med. Assoc.*, **199**, 519 (1967).
- ⁹ Gerber, P., Whang-Peng, J., and Monroe, J. H., *Proc. US Nat. Acad. Sci.*, **63**, 740 (1969).
- ¹⁰ Flamm, W. G., Bond, H. E., and Burr, H. E., *Biochim. Biophys. Acta*, **129**, 310 (1966).
- ¹¹ Kit, S., Dubbs, D. R., and Hsu, T. C., *Virology*, **19**, 13 (1963).
- ¹² Kaplan, A. S., and Ben-Porat, T., *Virology*, **19**, 205 (1963).
- ¹³ Dulbecco, R., Hartwell, L. H., and Vogt, M., *Proc. US Nat. Acad. Sci.*, **53**, 403 (1965).
- ¹⁴ Hatanaka, M., and Dulbecco, R., *Proc. US Nat. Acad. Sci.*, **56**, 736 (1966).
- ¹⁵ Takahashi, M., Van Hoosier, G. L., and Trentin, J. T., *Proc. Soc. Exp. Biol. Med.*, **122**, 740 (1966).
- ¹⁶ Nakata, Y., and Bader, J. P., *Tenth Intern. Cancer Cong.*, 146 (1970).
- ¹⁷ Zur Hausen, H., and Schulte-Holthausen, H., *Nature*, **227**, 245 (1970).
- ¹⁸ Maurer, B. A., Imamura, T., and Wilbert, S. M., *Cancer Res.*, **30**, 2870 (1970).
- ¹⁹ Gerber, P., and Deal, D., *Proc. Soc. Exp. Biol. Med.*, **134**, 748 (1970).

Herpes-like Epstein-Barr Virus in Leprosy

THE herpes-like virus (HLV), first detected¹ in lymphoid cell cultures derived from Burkitt’s lymphoma tissue, has been suggested as the causative agent of diseases such as Burkitt’s lymphoma itself², infectious mononucleosis^{3,4}, carcinoma of the posterior nasal space⁵ and sarcoidosis⁶. It appears that between 70% and 85% of normal adults have antibodies against HLV^{7,8} suggesting that the agent is widespread, but

it is also clear that the pathogenic nature of the virus is uncertain⁹.

The role of cell-mediated immunity in host reactions to HLV infection has received little attention¹⁰, but the impairment of cellular immunity has been thought to result in increases in humoral antibody production^{11,12}. Leprosy, caused by *Mycobacterium leprae*, has two clinico-pathological forms: lepromatous, associated with impaired delayed hypersensitivity, and tuberculoid, with intact cutaneous reactivity^{12,13}. To determine the role of cellular immunity in host responses to HLV infection, we ascertained the anti-HLV antibody titres in patients with lepromatous and tuberculoid leprosy.

Twenty-two serum specimens from patients with leprosy (eleven each of the lepromatous and tuberculoid types), diagnosed by identification of the microorganism in pathological specimens and by skin testing in the leprosarium "Aghia Barbara" in Athens, Greece, were studied. The sera were tested by indirect immunofluorescence for the presence of antibody titres to HLV⁹. Aliquots of the same specimens were tested for the IgG immunoglobulin concentration by the quantitative radial immunodiffusion method of Mancini¹⁴ (using plates from Melpar Inc.). Normal adult values obtained in our laboratory for IgG are 600 to 1,800 mg/100 ml.

Table 1 summarizes the distribution of anti-HLV antibody titres among the lepromatous and tuberculoid patients, who all had antibody titres to the HLV. Seven of the eleven patients with lepromatous leprosy had anti-HLV antibody titres of 1:640 or higher, but only three of eleven patients with tuberculoid leprosy attained the concentration of 1:640. Using a Mann-Whitney U test, the concentrations of anti-HLV antibody were found to be significantly lower in patients with tuberculoid leprosy (one-tail test: $P=0.016$) than those with lepromatous leprosy.

Antibody to HLV is restricted to the IgG class of immunoglobulins⁹. Table 1 also compares the IgG immunoglobulin concentrations in the patients with their corresponding anti-HLV titres. There is no correlation between the two values. Some sera with high IgG concentrations had anti-HLV antibody titres as low as 1:40, while others with normal IgG concentrations had antibody titres as high as 1:1,280.

A cell-mediated immunological response seems to be the principal method by which the immunocompetent host restricts intracellular parasitism by microbial and viral agents and maintains surveillance for neoplastic cells. The lymphoid cells involved in the immunological recognition and response to these altered cells seem to release a variety of soluble factors which would destroy such abnormal cells (lymphotoxin) in non-specific ways and render surrounding tissues resistant to further viral infection (interferon). Furthermore, factors may be released which cause the influx of granulocytes (leucotactic factor) and macrophages (migration inhibitory factor), and recruit lymphoid cells by specific (transfer factor) and non-specific means (blastogenic factors) to increase the total

response¹⁵. The failure of these defensive reactions *in vivo* would be expected to decrease capacities to restrict cells containing intracellular parasites, leading to increased production of the infectious agent and its antigens, thus increasing the antigenic load on the antibody-forming system of the host. Impairment of cellular immunity *in vivo*, therefore, should be associated with concomitant increases in humoral antibody production. This antibody escape mechanism occurs in a number of clinical disorders with altered cell-mediated immune responses. Patients with sarcoidosis have a marked deficiency in delayed cutaneous allergy accompanied usually by high concentrations of circulating immunoglobulins and they respond to immunization with an overproduction of antibody¹¹. It has been shown¹² that patients with the lepromatous form of leprosy have impaired delayed cutaneous reactivity to lepromin, yet the quantities of circulating antibodies to antigens of *M. leprae* are increased. Patients with tuberculoid leprosy have intense delayed skin reactivity to lepromin although antibodies to mycobacterial antigens are less frequently detected in these cases¹³.

The findings reported here add to the evidence for an antibody escape mechanism, as indicated by the increased concentrations of IgG immunoglobulins in our patients with lepromatous leprosy.

Many viruses replicate better in lymphoblasts¹⁶. Possibly, lymphoreticular diseases, such as sarcoidosis and leprosy, favour the replication of latent viruses (for example HLV) contained within stem cells. Without adequate cell-mediated immune responses to restrict this replication, high anti-viral antibody titres should result. The presence of high anti-HLV antibody titres in lepromatous leprosy with impaired delayed-type hypersensitivity supports the thesis that cell-mediated immunity is a significant factor in host response to HLV, and that high anti-HLV antibody titres occur in diseases associated with impaired delayed-type hypersensitivity. This would explain the finding of high anti-HLV antibody in sarcoidosis⁷, a disease known to be associated with anergy and, perhaps also, Burkitt's lymphoma and carcinoma of the posterior nasal space. Anti-HLV antibody titre determination may be a useful alternative to skin testing to assess the status of cellular immunity in various clinical disorders.

We thank Dr Yashar Hirshaut and Bozena Dvorak for assistance and Dr Peter Workman for the statistical analyses. The work was supported by the National Cancer Institute (Special Virus Cancer Programme). Dr Glade has a USPHS research career development award.

P. S. PAPAGEORGIOU
C. SOROKIN
K. KOUZOUTZAKOGLU
P. R. GLADE

Mount Sinai School of Medicine,
Department of Pediatrics,
City University of New York,
New York, NY 10029

Received December 17, 1970.

Table 1 Distribution of Anti-HLV Antibody Titres and IgG Immunoglobulin Concentrations in the Patients with Lepromatous and Tuberculoid Leprosy

Lepromatous type			Tuberculoid type		
Patient	Anti-HLV titre	IgG concentration	Patient	Anti-HLV titre	IgG concentration
1	1:640	800	1	1:160	1,900
2	1:320	2,000	2	1:40	1,050
3	1:640	2,200	3	1:40	1,800
4	1:640	2,000	4	1:160	950
5	1:640	2,500	5	1:320	1,300
6	1:160	1,300	6	1:640	850
7	1:640	1,300	7	1:640	1,600
8	1:160	2,000	8	1:160	950
9	1:1,280	1,300	9	1:640	1,300
10	1:320	1,400	10	1:40	950
11	1:640	2,000	11	1:40	1,300

¹ Epstein, M. A., Achong, B. G., and Barr, Y. M., *Lancet*, i, 702 (1964).

² Henle, G., and Henle, W., *J. Bact.*, **91**, 1248 (1966).

³ Henle, G., Henle, W., and Diehl, V., *Proc. US Nat. Acad. Sci.*, **A**, **59**, 94 (1968).

⁴ Niederman, J. C., McCallum, R. W., Henle, G., et al., *J. Amer. Med. Assoc.*, **203**, 205 (1968).

⁵ Old, L. J., Boyse, E. A., Oehgen, H. F., et al., *Proc. US Nat. Acad. Sci.*, **56**, 1699 (1966).

⁶ Hirshaut, Y., Glade, P. R., Octavio, L., Vieiza, B. D., et al., *New Eng. J. Med.*, **283**, 502 (1970).

⁷ Levy, J. A., and Henle, G., *J. Bact.*, **9**, 275 (1966).

⁸ Gerber, P., and Birch, S. M., *Proc. US Nat. Acad. Sci.*, **58**, 478 (1968).

⁹ Hirshaut, Y., Glade, P. R., Moses, H., et al., *Amer. J. Med.*, **47**, 520 (1969).

¹⁰ Glade, P. R., and Hirschhorn, K., *Cell Immunol.*, **1**, 359 (1970).

¹¹ Sandi, J. H., Palmer, P. P., Mayock, R. L., et al., *Amer. J. Med.*, **19**, 401 (1955).

- ¹² Bullock, W. E., *New Eng. J. Med.*, **278**, 298 (1968).
¹³ Bunell, R. G., and Rheims, M. S., *Intern. J. Leprosy*, **25**, 233 (1957).
¹⁴ Mancini, G., Vaerman, J. P., Carbonara, A. O., et al., *Proc. Eleventh Colloquium, Bruges, 1963* (edit. by Peeters, H.), 370 (Elsevier, Amsterdam, 1964).
¹⁵ *Mediators of Cellular Immunity* (edit. by Lawrence, H. S., and Landy, M.) (Academic Press, New York, 1969).
¹⁶ Glade, P. R., Paltrowitz, I. M., and Hirschhorn, K., *Bull. NY Acad. Med.*, **45**, 647 (1969).

Frequency of Polyploid Spermatozoa in Man

DIPLOID spermatozoa have been found in mouse, rabbit, bull (reviewed by Beatty¹) and man^{2,3}. In the rabbit they form 0.03% of the total sperm and in the bull 0.1% to 0.17%, but the incidence in man is not known. Because such sperm could well be involved in the formation of triploid embryos, which are almost invariably aborted in man⁴, knowledge of their frequency would be of interest.

Of 1,670 sperm measured in this study, sixteen were diploid, and one was tetraploid, giving 1.02% of polyploid sperm (Table 1). The mean optical density of the diploid sperm was 1.990 ± 0.070 times, and the mean area 1.578 ± 0.069 times, that of haploid sperm. These figures are very close to the expected values of 2.0 and 1.587 ($2^{2/3}$). The corresponding values for the single tetraploid sperm were 3.68 for density and 1.84 for area. The subjects studied were aged between 23 and 27 yr, and the proportions of polyploid sperm ranged from 0.74% to 1.53%, the highest proportion occurring in the youngest subject. Our data are, however, clearly inadequate to make any deductions about a possible effect of age.

If a diploid spermatozoon fertilizes a normal ovum, the resulting zygote would be triploid. Triploid embryos are almost invariably aborted, and form about 5% of spontaneous abortions⁵. Estimates of the spontaneous human abortion rate vary from about 15% to 27%, so that roughly 1% of all conceptions are triploid⁵. Boué *et al.*⁶ and Schindler and Mikamo⁷ considered the origin of human triploid embryos, and concluded that they result both from digyny, through suppression of the second polar body, and diandry. Penrose and Delhanty⁸ showed that a triploid abortus had resulted from digyny, two

Table 1 Characteristics of the Sperm Samples used and Frequency of Polyploid Sperm

Subject	Age	Sperm count * $\times 10^{-6}$ ml. ⁻¹	Sperm motility * (%)	Sperm morphology * % normal	Number of sperm measured	Number diploid	Number tetraploid	% Polyploid
1			Data not available		450	3	1	0.89
2	26	81	75	59	408	3	0	0.74
3	27	44	69	73	419	4	0	0.95
4	23	35	60	52	393	6	0	1.53
Total	—	—	—	—	1,670	16	1	1.02

* The following values are regarded as normal: sperm count, above 30×10^6 ml.⁻¹; sperm motility, above 50%; sperm morphology, above 55%.

Ejaculated sperm were obtained from men attending a sub-fertility clinic; all were chromosomally normal, and their sperm counts, percentage of normal sperm and sperm motility were within normal limits (Table 1). Except for subject 2, a drop of whose semen was smeared on a slide and fixed for 30 min in methanol-acetic acid (3:1), samples of semen were fixed in suspension in three changes of methanol-acetic acid, and a drop of the resulting suspension dropped onto a slide and allowed to spread. This technique gave more uniform results with Feulgen's method than smearing before fixation. To measure the DNA content of the sperm, Feulgen's method was used on the slides, hydrolysing in 5 M hydrochloric acid at room temperature for 1.5 h. Slides were subsequently mounted in DPX. A Vickers M85 microdensitometer was used to measure the integrated optical density and the area of the sperm; three measurements of each parameter were made for each sperm, and we used the means of these values in subsequent calculations. The slides were sampled at regular intervals (usually 2 mm in each direction) and all sperm within the field of the instrument were measured until approximately 400 had been measured from each subject; these occupied about half the slide in each case.

Because there was considerable variation in the integrated optical density and the area of the sperm, an objective criterion of diploidy was used. Sperm were regarded as diploid if their densities were more than 1.64 standard deviations (95% limit) above the mean value, and if half their density was within the normal range of all the sperm measured; a similar criterion was used for the areas, except that the area divided by $2^{2/3}$ was required to fall within the normal range (because area is a measure of only two of the three dimensions which should be increased in a larger sperm). In fact, almost all the polyploid sperm found by measurement were easily recognizable visually by their larger areas and greater densities.

of the three sets of chromosomes resembling the mother's. Schindler and Mikamo⁷ regarded double fertilization (by two haploid sperm) as the only likely mechanism of diandry, and stated that there is no evidence that diploid sperm are capable of successful fertilization. Nevertheless, as there is a significant proportion of diploid sperm in human semen (between 6 and 30 times greater than that reported in other mammals¹) it seems unwise to ignore them as a possible cause of triploid embryos. Even if they are capable of fertilization, however, there is clearly some selection against them, because their frequency is comparable with that of triploid embryos, many of which are formed by other mechanisms. It seems, therefore, that diploid sperm are almost as frequent as 24 YY sperm³ and are similarly less efficient than normal haploid sperm in fertilization.

I thank Mr P. Edmond and Sister M. Isdale for sperm samples, Professor H. J. Evans and Miss K. Buckton for advice, and Mrs Sheila Christie and Mrs Lily Gowans for technical assistance.

A. T. SUMNER

*MRC Clinical and Population Cytogenetics Unit,
Western General Hospital,
Edinburgh EH4 2XU*

Received February 12, 1971.

- ¹ Beatty, R. A., *Biol. Rev.*, **45**, 73 (1970).
² Patau, K., *Pathol. Biol.*, **11**, 1163 (1963).
³ Sumner, A. T., Robinson, J. A., and Evans, H. J., *Nature New Biology*, **229**, 231 (1971).
⁴ Carr, D. H., *Amer. J. Obstet. Gynec.*, **104**, 327 (1969).
⁵ Carr, D. H., in *Human Population Cytogenetics* (edit. by Jacobs, P. A., Price, W. H., and Law, P.), 103 (University Press, Edinburgh, 1970).
⁶ Boué, J. G., Boué, A., and Lazar, P., *Ann. Genet.*, **10**, 179 (1967).
⁷ Schindler, A. M., and Mikamo, K., *Cytogenetics*, **9**, 116 (1970).
⁸ Penrose, L., and Delhanty, J. D. A., *Lancet*, **i**, 1261 (1961).

Sex Difference in the Number of Adipose Cells from Genetically Obese Rats

It is well known that there is no increase in the number of adipose cells in many kinds of experimental obesity. In genetically obese mice, obob obese hyperglycaemic^{1,2} or yellow obese³ syndromes as well as in hypothalamic obesities produced by aurothioglucose in mice⁴ or stereotaxic lesions in rats⁵, there is only an enlargement of the adipose cells. The same results are obtained in rats made obese by a high fat diet⁶. There is only one known exception to this rule: obesity induced by a high fat diet in female mice⁷, in which the number of cells is increased by a factor of 2.5. Because male animals were used in all experiments except the last, the question arises whether there may be a sex difference in the adipose tissue development in obese animals.

Genetically obese rats (fafa)⁸ 2.5 months old and their litter mates, fed with my control diet, were used. Whole perigenital fat pads were weighed and treated as previously described⁶: portions of histological sections on slides, projected on the ground glass of a Projectina microscope, were counted for their adipose cells at a magnification of $\times 130$. The mean volume was calculated and the number of adipose cells estimated by the ratio of fat pad weight to mean adipose cell volume $\times 0.91$ (0.91 is the measured density of the pads).

Table 1 shows that the body weights of obese rats doubled in the females but increased less in the males. The weight of perigenital fat pads increased 3.2 and 9.7-fold in males and females respectively. Adipose cells enlarged 4.6 and 4.0-fold in obese males and females respectively. In male obese rats there were significantly fewer epididymal adipose cells than in their litter mates, but a 2.3-fold increase in their number was observed in the parametrial cells of the obese females.

Table 1 Weights of the Body and of the Two Perigenital Fat Pads of 2.5 Month Old Genetically Obese Male and Female Rats and Their Litter Mates

	Lean litter mates	Obese
Males		
Body weight (g)	276 $\pm$ 16.0	366 $\pm$ 17.3
Epididymal fat pad weight (g)	2.82 $\pm$ 0.260	9.00 $\pm$ 0.327
Adipose cell volume ($\times 10^3 \mu\text{m}^3$)	276 $\pm$ 25.5	1,275 $\pm$ 85.2
No. of adipose cells (millions)	9.34 $\pm$ 0.458	6.50 $\pm$ 0.256
Females		
Body weight (g)	165 $\pm$ 2.4	320 $\pm$ 13.6
Parametrial fat pad weight (g)	1.64 $\pm$ 0.189	15.9 $\pm$ 0.71
Adipose cell volume ($\times 10^3 \mu\text{m}^3$)	379 $\pm$ 46.6	1,536 $\pm$ 43.5
No. of adipose cells (millions)	4.08 $\pm$ 0.292	9.48 $\pm$ 0.460

The cell volume of the obese groups increased, but cell number increased only in the female group (2.3 times). All differences are highly significant ($P < 0.01$). Values are mean $\pm$ s.e. There were six animals in each of the four groups.

Hypertrophy of the adipose cells in the obese females could only account for a parametrial fat pad weight of $1.64 \times 4.0 = 6.64$ g—9.26 g less than the actual weight. And so about 70% of the weight of the pads was due to formation of new fat cells which were themselves hypertrophied.

These results clearly established a sex difference in the development of perigenital adipose tissue. They are in good agreement with the literature which records no increase in fat cell number in male obese rats or mice¹⁻⁶. But there was a marked hyperplasia in the parametrial adipose cells in the females which was very similar to those observed in female mice made obese by a high fat diet⁷.

Exceptions to the rule that only hypertrophy is observed in male adult animals had been claimed: there is hyperplasia of adipose cells in rats after repeated injections of insulin⁸⁻¹⁰ or meal feeds¹¹. In these animals there was no increase in the weight of the epididymal fat pad, and adipose cell volume seemed to be reduced; the differences, however, were very small. The principal effect, in the two cases, was a great increase in the total DNA content of the pads (+50% for meal fed rats¹¹ and +100% for rats treated with insulin¹⁰). Such differences cannot be the effect of a marked hyperplasia: the weight of fat pads was unchanged and cell volume very slightly smaller; so it can be concluded that there was an increased DNA content in stromal structures. Such an effect has been established in hypophysectomized rats after injections of somatotrophic hormone: the rate of DNA synthesis of supporting and vascular structures was enhanced, but there was no effect on division in adipose cells¹². In my rats, the histological appearance of perigenital adipose tissue was the same as that described for rats made obese by a high fat diet⁶. There was no difference, except for the size of the cells, between fat tissues of control and obese rats, although there was a marked and similar hyperinsulinism in males as in females in the obese group (to be published later).

I thank A. Alexia for technical assistance.

DANIEL LEMONNIER

Laboratory of Biology and Human Nutrition,
CNAM 292 Rue Saint Martin,
75 Paris 3

Received December 15, 1970.

- Hellman, B., Täljedal, I.-B., and Westman, S., *Acta Morphol. Neerl-Scand.*, **5**, 182 (1962).
- Lemonnier, D., Winand, J., Furnelle, J., and Christophe, J. (in the press).
- Hellman, B., Thelander, L., and Täljedal, I.-B., *Acta Anat.*, **55**, 286 (1963).
- Hellman, B., Täljedal, I.-B., and Petersson, B., *Med. Exp.*, **6**, 402 (1962).
- Hirsch, J., and Han, P. W., *J. Lipid Res.*, **10**, 77 (1969).
- Lemonnier, D., *Arch. Anat. Microsc. Morphol. Exp.*, **59**, 1 (1970).
- Lemonnier, D., *Experientia*, **26**, 974 (1970).
- Zucker, L. M., and Zucker, T. F., *J. Hered.*, **52**, 275 (1961).
- Hausberger, F. X., and Hausberger, B. C., *Anat. Rec.*, **127**, 305 (1957).
- Kazdova, L., and Vrana, A., *Horm. Metab. Res.*, **2**, 117 (1970).
- Braun, T., Kazdova, L., Fabry, P., Lojda, Z., and Hromadkova, V., *Metabolism*, **17**, 825 (1968).
- Hollenberg, C. H., and Vost, A., *J. Clin. Invest.*, **47**, 2485 (1968).

Polychlorinated Biphenyl absorbed from Sediments by Fiddler Crabs and Pink Shrimp

POLYCHLORINATED biphenyls (PCBs) are manufactured in the United States, Europe, and Japan and used as plasticizers, flame retardants, insulating and heat exchange fluids and in many other products¹. Structurally related to DDT, soluble in lipid but relatively insoluble in water, and extremely persistent in the environment², they have been reported in many marine and estuarine organisms³. One PCB, 'Aroclor 1254' (Monsanto), was reported in water, sediments, and biota from Escambia Bay, Florida⁴.

In August 1969, pink, white and brown shrimps (*Penaeus duorarum*, *P. setiferus*, and *P. aztecus*) from Escambia Bay were found to contain whole body residues of 'Aroclor 1254' at concentrations as high as 14.0 p.p.m. Most was concentrated in the hepatopancreas; residues in seven composite samples of at least five shrimps ranged from 0.6 to 120.0 p.p.m. In April 1970, fiddler crabs (*Uca minax*) collected at three stations along the lower Escambia River and upper Escambia Bay had individual whole body residues of 0.45 to 1.5 p.p.m.

To determine the distribution of 'Aroclor 1254' in the estuary, forty samples of water were collected in glass bottles; twenty sediments were collected with a core or dredge sampler, and many estuarine organisms were captured by trawl and dredge. These were analysed by gas liquid chromatography⁴. The largest accumulations of 'Aroclor' were found in the sediments. In one survey (February 1970), concentrations ranged from 0.6 to 61.0 p.p.m. in a sample taken at the discharge point of the industrial plant that accidentally released the chemical and at eleven stations extending 16 km downstream (Fig. 1). 'Aroclor' was not detected in sediments collected above the plant but soil samples from the bank near the mouth of the river 6.5 km downstream from the source had 1.4 to 1.7 p.p.m. of this chemical. Subsequent samplings from three stations in the Bay showed little change in the amounts of the chemical even after nine months. We therefore investigated whether shrimp and fiddler crabs could accumulate 'Aroclor 1254' from the sediments.

upper end of five aquaria inclined 10 degrees so as to simulate a natural environment. Ten crabs from a population which had no contamination were placed in each aquarium with one serving as a control. Water with an average salinity of 27 p.p.t. at 13° C flowed at 5 l./h through the lower end. Both shrimp and crabs were fed fish containing <0.03 p.p.m. 'Aroclor 1254' each day for 30 days. Five crabs were removed from each aquarium, killed and rinsed with a 50% mixture of acetone and water to remove 'Aroclor 1254' from material adhering to the exoskeleton. To ensure enough tissue for analyses, individual crabs and pooled samples of hepatopancreas from shrimp were mixed with anhydrous Na₂SO₄ and extracted with petroleum ether in a Soxhlet apparatus; the extract was purified with 'Florisil'⁵. 'Aroclor 1254' was quantitated by gas liquid chromatography⁴. The ratio of individual 'Aroclor' isomers maintained integrity in the sediments and tissues of test animals throughout the investigation.

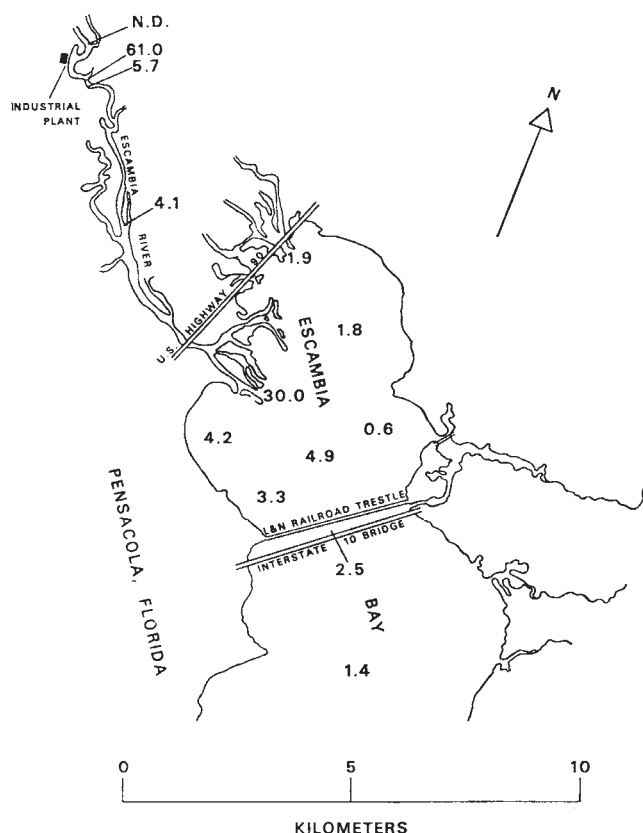


Fig. 1 Study area showing lower Escambia River, upper Escambia Bay and stations where sediment was collected on February 24, 1970. The amount of 'Aroclor 1254' found at each station is given in parts per million (p.p.m.). ND, Not detected.

Table 1 'Aroclor 1254' in Individual Fiddler Crabs and Hepatopancreas from Pink Shrimp

Sediment	'Aroclor 1254' in sediment (p.p.m. dry weight)	Average and range of 'Aroclor 1254' in five individual fiddler crabs (p.p.m. wet weight)	'Aroclor 1254' in combined hepatopancreas from four or more shrimp (p.p.m. wet weight)
Sandy silt *	61.0	80.0 ± 25.0	240.0
Silt †	30.0	17.0 ± 9.0	6.1
Sandy silt *	5.7	—	9.8
Silt †	4.9	3.6 ± 0.9	1.3
Sandy silt *	4.1	—	6.7
Silt †	2.5	3.2 ± 0.9	1.1
Silt †	1.4	—	0.2
Sand control *	ND	0.3 ± 0.2	< 0.2
Silt control †	ND	—	< 0.2

The samples of crab and shrimp were kept for 30 days on naturally occurring sediments containing 'Aroclor'. Mortalities occurred in some groups of shrimp which could not be attributed to 'Aroclor 1254'.

* Sediment which contained particles of sand > 354 µm in diameter.

† Sediment which contained particles of sand 354 µm or less.

—, No sample.

ND, Not detectable.

Fiddler crabs and shrimp exposed to the contaminated sediments accumulated 'Aroclor 1254' in their tissues by ingesting contaminated sediment particles or by absorbing the leached chemical from water. They sifted the sediments before ingesting the particles. In most cases, the amount of 'Aroclor' in individual crabs or in shrimp hepatopancreas was directly related to the amount in the sediments. We analysed the distribution of the chemical in the sediment in relation to particle size and found it was evenly distributed. Large concentrations of 'Aroclor' residues may also accumulate in the hepatopancreas of shrimp exposed to sandy silts because the chemical leaches from the sediments and is absorbed through the gills. Three and a half parts per billion (p.p.b.) were in the effluent water from the aquarium with sandy silt (61.0 p.p.m. 'Aroclor'); the water from the silt (30.0 p.p.m.) had only 0.5 p.p.b. Even though fiddler crabs were not continuously covered with water, they could have obtained 'Aroclor' from the water as they wetted their gills.

We have demonstrated experimentally that 'Aroclor 1254' can enter the estuarine food chain from sediments. Others⁴ have identified this chemical in higher trophic life such as fish, and since PCBs are widespread in the world³, we should

Seven contaminated sediments (Table 1) were collected with a Petersen dredge from upper Escambia Bay and lower Escambia River at depths of 0.6 to 9 m. Beach sand and additional samples of sediment collected upstream from the contaminated section of the river served as uncontaminated controls. Four kilograms of each of the sediments and ten adult pink shrimp (*P. duorarum*) from a population which had previously shown no contamination with 'Aroclor' were placed in each of nine aquaria. Water at 17° C flowing at a rate of 12 l./h, with an average salinity of 27 parts per thousand (p.p.t.), covered the substratum to a depth of 10 cm.

Fiddler crabs (*U. pugilator*) were exposed to 'Aroclor' by placing them on five sediment substrates collected as described. Four kilograms of each of the sediments were placed in the

become more alert to the presence, movement and effects of these chemicals in the environment.

D. R. NIMMO
P. D. WILSON
R. R. BLACKMAN
A. J. WILSON, JUN.

US Environmental Protection Agency,
Gulf Breeze Laboratory,
Gulf Breeze, Florida 32561

Received September 17; revised December 10, 1970.

- ¹ Reynolds, L. M., *Bull. Envir. Contam. and Toxicol.*, **4**, 128 (1969).
- ² Risebrough, R. W., and Brodine, V., *Environment*, **12**, 16 (1970).
- ³ Risebrough, R. W., Rieche, P., Peakall, D. B., Herman, S. G., and Kirven, M. N., *Nature*, **220**, 1098 (1968); Koeman, J. H., Ten Noever De Brauw, M. C., and De Vos, R. H., *Nature*, **221**, 1126 (1969); Jensen, S., Johnels, A. G., Olsson, M., and Otterlind, G., *Nature*, **224**, 247 (1969).
- ⁴ Duke, T. W., Lowe, J. I., and Wilson, jun., A. J., *Bull. Environ. Contam. and Toxicol.*, **5**, 171 (1970).
- ⁵ Mills, P. A., Onley, J. H., and Gaither, R. A., *J. Assoc. Offic. Agric. Chem.*, **46**, 186 (1963).

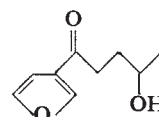
A Lung Oedema Factor from Mouldy Sweet Potatoes (*Ipomoea batatas*)

WE have already described the toxic properties of sweet potato tubers infected with common moulds^{1,2}. The toxic properties are not directly attributable to the fungi because poisons were not found when the organisms were grown in autoclaved sweet potato slurry. The experimental infection of viable sweet potato slices or of the intact tubers nevertheless resulted in the production of many furanoterpenoid compounds, some of which are markedly toxic for laboratory and commercial animals.

As well as the previously recognized hepatotoxins ipomeamarone [(+)-ngaione]³ and ipomeamaronol², we have also isolated a more potent toxin called lung oedema factor (LO) from ether and chloroform extracts of both artificially and naturally contaminated sweet potatoes⁴, which recalls that natural outbreaks of mouldy sweet potato poisoning in cattle all describe respiratory distress related to lung oedema as the principal sign of disease^{4,5}. On the other hand, hepatotoxicity which might be attributable to ipomeamarone or ipomeamaronol has not been reported in most enzootics.

Fresh tuber slices inoculated with sweet potato slurry-shake cultures of *Fusarium javanicum* and incubated for approximately 6 days at 22°–24° C contained significant quantities of all three toxins. We partially purified these metabolites by partitioning crude ether extracts of infected sweet potatoes on silica gel columns using elution with a hexane-ethyl acetate gradient. The column eluates were monitored by thin-layer chromatography (TLC) (silica gel; 9 : 1 benzene-methanol). Ehrlich's reagent produced twelve to fifteen coloured spots representing furanoterpenoids on TLC strips developed from the crude ether extract. Preliminary toxicity assays in mice showed that the LO factor was associated with a reddish-purple spot at $R_f=0.4$ which contained numerous compounds by gas chromatographic analysis.

Column eluate enriched with LO, consisting chiefly of the reddish-purple TLC spot material, was purified further by preparative gas-liquid chromatography (GC) of trimethylsilylated (TMS) product. A single GC peak corresponding to the TMS ether of lung toxic material was obtained. We removed the TMS group by acid hydrolysis to give pure toxin, a colourless oil with a pungent odour. Spectral evidence (Table 1) indicates that its structure is 1-(3'-furyl)-4-hydroxy-1-pentanone.



We propose the trivial name, 4-ipomeanol, for this compound which is a dihydro derivative of ipomeanine, 1-(3'-furyl)-1,4-pentanedione, another known metabolite of sweet potatoes⁶. There are no reports indicating toxicity for ipomeanine.

The empirical formula of 4-ipomeanol, $C_9H_{12}O_3$ (molecular weight 168), was deduced from the mass spectrum by exact mass measurement of the $P-H_2O$ ion. (We measured a value of 150.070 ± 0.002 ; $C_9H_{10}O_2$ requires a value of 150.068.) The β -furyl keto moiety was indicated by the characteristic nuclear magnetic resonance (NMR) signals for the furyl protons⁷, by the stretching frequency of the conjugated carbonyl group, by the ultraviolet spectrum⁸ and by the intense m/e 95 ion (β - $C_4H_3O-CO^+$). The third oxygen function is a hydroxyl group (ν 3,390 cm^{-1}). Double irradiation NMR showed that the hydroxyl group was embodied in a methyl carbinol. The chemical shifts of the remaining two methylene groups were consistent with the unbranched form of the structure.

Table 1 Spectra of 4-Ipomeanol

Infrared, cm^{-1} (neat)	Ultraviolet nm (cyclohexane)	NMR, δ p.p.m. (CCl_4)	MS, m/e (%)
3,480 (OH)	243 (ϵ 3,000)	1.15 (3H, d, $J=6Hz$, 5- CH_3)	168 (5) Molecular ion
3,110 (furyl)	211 (ϵ 5,100)	1.67 (2H, m, 3- CH_2)	153 (2) M- CH_3
2,940		2.50 (1H, broad s, OH)	150 (6) M- H_2O
2,900		2.83 (2H, t, $J=7Hz$, 2- CH_2)	124 (5) M- CH_3 -HCO
1,675 (conj. C=O)		3.73 (1H, m, 4-CH)	121 (2) M- H_2O -HCO
1,560 (furyl)		6.75 (1H, m, 4'-CH)	110 (47)
1,510 (furyl)		7.42 (1H, m, 5'-CH)	95 (100) furyl-C=CH ₂ furyl-CO
1,160 880 (furyl)		8.07 (1H, m, 2'-CH)	

4-Ipomeanol is acutely toxic to mice, producing the lung oedema and pleural effusion previously observed with crude sweet potato extracts. Doses of toxin (1–6 mg) given intraperitoneally to mice produced immediate signs of general illness, followed quickly by dyspnoea which gradually increased in severity to the time of death, apparently from anoxia, in 5 to 8 h. Comparable quantities given orally were usually followed by a short period of apparent latency (2 to 3 h) followed by increasing respiratory distress leading to death within 24 h.

At post-mortem examinations, clear fluid, sometimes as much as 1 ml. or more, dripped from the nostrils when the animal carcass was held with its head downwards. The lungs were frequently congested and surrounded by 1–2 ml. of clear pleural fluid. Microscopic sections of the lungs stained with haematoxylin and eosin showed intra-alveolar oedema and congested blood vessels. In some animals there was a slight increase in abdominal fluid but we observed no significant pathological changes in abdominal viscera.

We have also isolated an isomer of 4-ipomeanol, tentatively assigned the structure 5-(3'-furyl)-5-hydroxy-2-pentanone, which is under investigation.

Sweet potatoes offered for sale in food stores often contain ipomeamarone, ipomeamaronol and 4-ipomeanol¹. The exact

nature of the apparent minimal damage which produces toxin in these tubers (that is whether it is microbial infection, exogenic chemical irritation, or mechanical damage) is not known. Because the usual cooking processes for sweet potatoes do not eliminate the toxins from contaminated tubers, possible implications for human health must now be considered and assessed.

We thank Dr Stanford L. Smith for NMR data. This work was supported by a National Institutes of Health grant and a Centre in Toxicology grant to Vanderbilt University.

B. J. WILSON
M. R. BOYD
T. M. HARRIS*
D. T. C. YANG†

*Division of Toxicology,
School of Medicine,
Vanderbilt University,
Nashville, Tennessee 37203*

Received November 23, 1970.

*Present address: Department of Chemistry, Vanderbilt University.

†Present address: Chemistry Department, University of Arkansas, Little Rock, Arkansas.

¹ Wilson, B. J., Yang, D. T. C., and Boyd, M. R., *Nature*, **227**, 521 (1970).

² Yang, D. T. C., and Wilson, B. J., *Phytochemistry* (in the press).

³ Hegarty, B. F., Kelly, J. R., Park, R. J., and Sutherland, M. D., *Austral. J. Chem.*, **23**, 107 (1970).

⁴ Hansen, A. A., *North Amer. Vet.*, **9**, 31 (1928).

⁵ Monlux, W., Fitte, J., Kendrick, G., and Dubisson, H., *South-western Vet.*, **6**, 267 (1953).

⁶ Kubota, T., and Ichikawa, N., *Chem. and Ind.*, **29**, 902 (1954).

⁷ Gronowitz, S., Sörlin, G., Gestblom, B., and Hoffman, R. A., *Arkiv. Kemi.*, **19**, 483 (1962).

⁸ Matsuura, T., Naya, K., Ischikawa, N., and Kubota, T., *Bull. Chem. Soc., Japan*, **35**, 1695 (1962).

Do Artificial Sweeteners ingested in Pregnancy affect the Offspring?

RATS treated with approximately 20 mg/kg of cyclamate each day have been found to behave abnormally¹ and it is believed that the behavioural changes may be induced during meiosis and embryonic development². The offspring are hyperactive and slow to develop a response to food reward and, once trained, they are deficient, in comparison with controls, in tasks requiring response inhibition. Our results suggest similarities to certain types of brain-lesioned rats and to minimal brain-damaged human offspring. We have studied the effects of artificial sweeteners on human offspring because, in the past decade, they have been consumed by vast numbers of women, particularly during pregnancy. The questionnaire technique, with all its inherent faults, has been used because this was the only method readily available; the area of interest was "hidden" in the series of questions asked. The data suggest that during the past decade there were correlations between the known use of artificial sweeteners during pregnancy and behavioural and physical aberrations in human offspring.

Questionnaires were returned by 975 women in the State of Massachusetts who had given birth to normal children and by members of associations for the mentally retarded who had given birth to a total of 247 mentally retarded children. Although our "normal" sample contains more young mothers than the mentally retarded sample, maternal age does not seem to be a significant factor in the use of artificial sweeteners. On the other hand, because artificial sweeteners became popular from the end of the 1950s to the end of the 1960s, a comparison between the two groups is only meaningful if children of the

same age are compared. The data we obtained show a statistically significant difference between the ratios of women from the two groups ingesting artificial sweeteners during the past decade: in the normal group, usage increased from 11.5% during 1959–61 (nine out of seventy-eight pregnancies) to 14.5% during 1962–64 (35 out of 242) to 21.5% during 1965–69 (141 out of 655); in the mentally retarded group the corresponding figures are 17.7% (14 out of 79), 22.6% (26 out of 115) and 35.8% (19 out of 53). For the period 1962–4, $\chi^2 = 4.75$, d.f. = 1, $P < 0.05$; and for 1965–69, $\chi^2 = 4.96$, d.f. = 1, $P < 0.05$.

Of the fifty mothers who ingested artificial sweeteners, twenty-nine had offspring with Down's syndrome. Obviously, larger numbers must be studied, but our data indicate that ingestion of artificial sweeteners is equally related to both Down's syndrome and to other forms of mental retardation. Whether this reflects action during meiosis, suggesting ingestion of the materials before fertilization, or action during embryonic development resulting in chromosomal mosaics, remains to be seen.

We had to determine by questioning their parents whether the normal children were completely normal or whether they had physical and behavioural problems. The answers could not be verified but it was known that many of the parents had sought medical advice. The results do suggest a possible connexion between ingestion of artificial sweeteners before and during pregnancy and the incidence of abnormalities in the non-mentally retarded group of children. The overall incidence of behavioural problems reported was 5.4% (that is, ten children out of 185) in offspring of the users and 2.0% (sixteen out of 790) in those of the non-users. The mothers described the most common problems as hyperactivity, nervous irritability, and extreme nervousness. These occurred in 0.6% (5 out of 790) of children from mothers not using artificial sweeteners, and in 4.3% (8 out of 185) of the children of mothers who did.

Of the physical anomalies reported, apart from flat feet, allergies, eczema and bronchitis (incidences of 2.9% in the children of users and 2.2% in those of non-users), the children of mothers who did not consume artificial sweeteners had an overall incidence of 4.4% (35 children out of 790) in comparison with 9.7% (18 out of 185) of the children of the users. The most common defects reported were deformities of the bones and joints of the hip, leg and foot; in this category the incidence in children of non-users was 1.5% (12 out of 790); that in children of users was 4.8% (9 out of 185). There was no overlap between the children reported to have physical or behavioural anomalies.

Our results are preliminary and do not demonstrate causal relationships in the human. But in the light of the effects of cyclamate on the behaviour of rat offspring, the implications of these studies make clear the need for further investigation.

This research was supported by the Dementia Praecox Grant, Department of Mental Health, Massachusetts, the International Sugar Research Foundation and the Henry Hittleson Family Foundation, New York. We thank Mary Ellen Fitts for assistance.

DAVID STONE
EDWARD MATAŁKA
BARBARA PULASKI*

*Worcester Foundation for Experimental Biology,
Shrewsbury,
and*

*Worcester State Hospital,
Worcester, Massachusetts*

Received November 10, 1970.

* Present address: Boston College, Boston, Massachusetts.

¹ Stone, D., Matalaka, E., and Riordan, J., *Nature*, **224**, 1326 (1969).

² Stone, D., and Matalaka, E., *Fourth Intern. Symp. Cybernetics: Cybernetics and the Management of Ecological Systems, Washington DC* (in the press).

Fusaric (5-Butylpicolinic) Acid, an Inhibitor of Dopamine β -Hydroxylase, affects Serotonin and Noradrenaline

THE principal biochemical defect associated with Parkinsonism is a marked decrease in the dopamine and serotonin content of the brain¹. L-Dopa, a precursor of dopamine, has been found to help patients suffering from this disease, presumably by increasing the concentration of dopamine in the brain².

We reported fusaric acid to be a potent, non-toxic inhibitor of dopamine β -hydroxylase *in vivo* and *in vitro*^{3,4} and originally applied this drug to hypertensive patients⁵. Recently, we have also attempted to treat Parkinsonism with fusaric acid and found this drug to be very effective in the relief of tremor, rigidity and speech. Fusaric acid and L-dopa, when administered together, were much more effective than either given alone. The therapeutic dose of L-dopa could be reduced to about 1 g per day in the presence of fusaric acid⁶. Although fusaric acid is a potent inhibitor of dopamine β -hydroxylase, it did not alter the cerebral dopamine concentrations⁴. We examined brain serotonin concentrations after the administration of fusaric acid in an attempt to understand the mechanism of its palliative action on Parkinsonism.

by fusaric acid (Table 1). Thus, fusaric acid prevented the decrease of brain serotonin caused by the administration of L-dopa and L-dopa prevented the depletion of noradrenaline by fusaric acid.

Serotonin may be accumulated by catecholaminergic neurones in vas deferens¹³ and in the pineal gland^{14,15}. These data are in accordance with histochemical findings that intraperitoneally administered serotonin is accumulated by dopaminergic neurones in the brain¹⁶, and that intraventricularly administered serotonin is accumulated in part by catecholaminergic neurones in the caudate nucleus¹⁷.

The accumulation of serotonin in synaptosomes from the hypothalamus and striatum of the rat is known to be inhibited by catecholamines¹⁸ and catecholamines are potent inhibitors of striatal serotonin accumulation¹⁹. Therefore, the increase of serotonin concentrations in rat brain after fusaric acid injection may be effected by depletion of noradrenaline. This hypothesis is supported by the following facts: (1) an increase of serotonin invariably correlates with a depletion of noradrenaline (Table 1), and (2) increases of serotonin after fusaric acid injection are smaller than those following the administration of MAO inhibitors, a potent example of which, pargyline (75 mg kg⁻¹), doubled serotonin concentrations, and this increase was not affected by fusaric acid (Berkowitz, Spector and H. H., unpublished data). Noradrenaline concen-

Table 1 Effect of Fusaric Acid on Concentration of Biogenic Amines in the Brains of Rats

Fusaric acid (75 mg kg ⁻¹)	No. of determinations	NA $\mu\text{g/g}^{-1}$ of brain	% of change	Serotonin $\mu\text{g/g}^{-1}$ of brain	% of change	Dopamine $\mu\text{g/g}^{-1}$ of brain	% of change
Control	14	0.42 $\pm$ 0.02	0	0.43 $\pm$ 0.02	0	0.69 $\pm$ 0.05	0
Single injection	12	0.31 $\pm$ 0.02 *	-25	0.49 $\pm$ 0.02 *	+13	0.70 $\pm$ 0.06	0
Three injections	12	0.25 $\pm$ 0.01 †	-40	0.54 $\pm$ 0.02 †	+25	0.67 $\pm$ 0.08	0
Three injections plus L-dopa	11	0.39 $\pm$ 0.02	-7	0.40 $\pm$ 0.03	-7	1.26 $\pm$ 0.09 †	+82
L-Dopa alone	11	0.44 $\pm$ 0.03	+6	0.29 $\pm$ 0.03 †	-33	1.20 $\pm$ 0.08 †	+74

Each value represents the mean $\pm$ s.e.

* Significant difference ($P < 0.05$) from control.

† Significant difference ($P < 0.005$) from control.

Fusaric acid (75 mg kg⁻¹) was administered one or three times at 20 h intervals, intraperitoneally. L-Dopa (200 mg kg⁻¹) was injected intraperitoneally 2 h after the last injection of fusaric acid. Rats were killed 3 h after the last injection of fusaric acid or 1 h after the administration of L-dopa.

Male Wistar rats (200 g) were injected intraperitoneally with fusaric acid (75 mg kg⁻¹). The concentrations of various amines were determined 3 h after the last injection of fusaric acid⁷⁻⁹, which did not interfere with the assays.

After three injections of fusaric acid the concentration of cerebral serotonin increased by 25% ($P < 0.005$) while that of noradrenaline decreased by 40% ($P < 0.005$) (Table 1).

Monoamine oxidase (MAO) activity¹⁰ and concentrations of 5-hydroxyindoleacetic acid (5-OHIAA) were measured in controls and after the administration of fusaric acid. There was no significant effect on MAO activity or the concentration of 5-OHIAA, compared with control values (Table 2). Therefore the increase of cerebral serotonin⁵⁻¹¹ after administration of fusaric acid was not caused by inhibition of MAO.

As the concentration of brain serotonin has been reported to decrease markedly in proportion to the dose of L-dopa administered¹², we determined the concentration of serotonin in the brains of rats treated with L-dopa and with L-dopa plus fusaric acid. When the two were administered sequentially, the concentrations of serotonin and noradrenaline were not significantly different from those of uninjected animals (Table 1), and furthermore, the increase in dopamine concentration after injections of L-dopa was not significantly affected

trations were not significantly different from control values when pargyline and fusaric acid were administered sequentially (Berkowitz, Spector and H. H., unpublished data). The failure of fusaric acid to alter the concentration of dopamine suggests that this is not a simple inhibition of dopamine β -hydroxylase. Dopamine may be degraded by O-methylation or oxidative deamination rather than by conversion to noradrenaline.

Table 2 Effect of Fusaric Acid on Concentration of Brain 5-Hydroxyindoleacetic Acid and Monoamine Oxidase Activity

Injection	5-Hydroxyindoleacetic acid $\mu\text{g g}^{-1}$ brain	Monoamine oxidase *
Control	0.35 $\pm$ 0.02	3.82 $\pm$ 0.10
Fusaric acid	0.40 $\pm$ 0.03	3.64 $\pm$ 0.08

* Values are expressed as μmol kynuramine disappeared per hour per g of brain. Fusaric acid (75 mg kg⁻¹) was administered 3 times at 20 h intervals and rats were killed 3 h after the last injection. Monoamine oxidase activity was determined in the brain homogenate according to Weissbach *et al.*¹⁰. Six rats were used for each determination.

These data suggest that fusaric acid may alleviate Parkinsonism by increasing brain serotonin. When fusaric acid and L-dopa are administered sequentially, the fusaric acid may prevent depletion of serotonin. This may be why fusaric acid with L-dopa is more effective than without in the treatment of this disease.

These data illustrate the hazard of correlating therapeutic efficacy of drugs used in the treatment of Parkinsonism with their effect on the concentration of brain dopamine.

This work was supported in part by a research grant from Hoffmann-LaRoche. I thank Drs S. Spector, B. Berkowitz, S. Bondy and J. H. Austin for discussions and Mr Tom Wiseley for technical assistance.

HIROYOSHI HIDAKA

Division of Neurology,
University of Colorado Medical Center,
Denver, Colorado

Received January 29, 1971.

* Present address: Institute of Biochemistry, Faculty of Medicine, University of Nagoya, Japan.

- ¹ Bernheimer, H., Birkmayer, O., and Hornykiewicz, *Klin. Wochenschrift*, **20**, 1056 (1961).
- ² Cotzias, G. C., Van Woert, M. D., and Schitter, L. M., *New Engl. J. Med.*, **276**, 374 (1967).
- ³ Hidaka, H., Nagatsu, T., Takeya, K., Takeuchi, T., Suda, H., Kojiri, K., Matsuzake, M., and Umezawa, H., *J. Antibiotics*, **22**, 228 (1966).
- ⁴ Nagatsu, T., Hidaka, H., Kuzuya, H., Takeya, K., Umezawa, H., Takeuchi, T., and Suda, H., *Biochem. Pharmacol.*, **19**, 35 (1970).
- ⁵ Terazawa, F., and Kameyama, M., *Jap. Circulation J.*, **35**, No. 349 (1971).
- ⁶ Okada, T., Fujita, T., Ota, O., Shinoda, T., Hidaka, H., Nagatsu, T., Takeuchi, T., and Umezawa, H., *Igaku No Ayumi*, **76**, 495 (1971).
- ⁷ Anton, A. H., and Sayre, D. F., *J. Pharmacol. Exp. Ther.*, **138**, 360 (1962).
- ⁸ Anton, A. H., and Sayre, D. F., *J. Pharmacol. Exp. Ther.*, **145**, 326 (1964).
- ⁹ Wise, C. D., *Anal. Biochem.*, **19**, 2517 (1967).
- ¹⁰ Weissbach, H., Smith, T. E., Daly, J. W., Witkop, B., and Udenfriend, S., *J. Biol. Chem.*, **235**, 1160 (1960).
- ¹¹ Udenfriend, S., Weissbach, H., and Brodie, B. B., *Methods of Biochemical Analysis*, 95 (Interscience, New York, 1958).
- ¹² Ng, K. Y., Chase, T. N., Colburn, R. W., and Kopin, I. J., *Science*, **170**, 76 (1970).
- ¹³ Thoa, N. B., Eccleston, D., and Axelrod, J., *J. Pharmacol. Exp. Ther.*, **169**, 68 (1969).
- ¹⁴ Bertler, A., Flack, B., and Owman, C., *Acta Physiol. Scand.*, **63**, Suppl. 239, 1 (1964).
- ¹⁵ Neff, N. H., Barret, R. E., and Costa, E., *Europ. J. Pharmacol.*, **5**, 348 (1969).
- ¹⁶ Lichtenstiger, W., Mutner, U., and Langemann, H., *J. Neurochem.*, **14**, 489 (1967).
- ¹⁷ Fuxe, K., and Ungerstedt, U., *J. Pharmac. Pharmacol.*, **19**, 335 (1967).
- ¹⁸ Iversen, L. L., in *Advances in Biochemical Pharmacology*, **2** (edit. by Costa, E., and Ciacobini, E.) (Raven Press, New York, 1970).
- ¹⁹ Shaskan, E. G., and Snyder, S. H., *J. Pharmacol. Exp. Ther.*, **175**, 404 (1970).

Mechanism of Insecticide-induced Diuresis in *Rhodnius*

WATER conservation is critical for insects because of their large surface area relative to volume and the generally arid environment in which they live. Their survival depends on the water-proofing mechanism of the cuticle and the control of excretory and respiratory mechanisms which lead to water loss. There are indications that the critical water balance is disrupted by

poisoning with certain insecticide chemicals¹⁻⁴, but the physiological basis for this action has not previously been defined. Treatment of *Rhodnius* with DDT results in the accumulation of a large volume of fluid in the rectum¹. We have now established that paralytic doses of compounds, representing each of the major types of insecticide chemicals, initiate massive excretion of fluid into the rectum of *Rhodnius*. Furthermore, the underlying mechanism involves the release of diuretic hormone, possibly from a bound form in the central nervous system, into the haemolymph where it contacts the Malpighian tubules and greatly increases their rate of secretion.

Forty-two insecticide chemicals and related compounds were administered, by topical application or injection, to fifth stage larvae of *Rhodnius prolixus* Stål after injection with 15 µl. of saline⁵ and sealing the anus to prevent the loss of rectal fluid. Twenty-one of the compounds caused no accumulation of fluid in the rectum and no paralytic symptoms, eight caused moderate to severe incoordination and some secretion of fluid into the rectum, and thirteen resulted in both paralysis and the accumulation of large volumes of rectal fluid in 18 h. Among the third group are DDT, γ-hexachlorocyclohexane, aldrin, dicrotophos, 'Zectran' and amino-'Zectran' (4-amino,3,5-xylyl methylcarbamate), allethrin, and nicotine, representatives of various types of insecticide chemicals which act initially on the nervous system to disrupt its function by several different mechanisms. One insecticide chemical, 'Zectran', was selected for more detailed investigation because it is fast acting and effectively poisons *Rhodnius* when given in low doses. When various doses of 'Zectran' were administered topically or by injection, large volumes of fluid accumulated only in the recta of insects which also showed paralytic symptoms. Furthermore, after topical application of 'Zectran', fluid secretion into the rectum was initiated at approximately the time when paralytic symptoms appeared. The excretion induced by insecticide chemicals is always initiated during the paralytic stage of poisoning. Insects injected with low doses of nicotine or the very toxic amino-'Zectran' (paralytic dose less than 0.1 µg/g) undergo temporary paralysis followed by apparent recovery, but they are in a desiccated condition because of the diuresis occurring during the paralysis.

The mechanism by which insecticide chemicals lead to the initiation of excretion was investigated by a technique involving determination of the secretion rate of isolated Malpighian tubules placed in 2 µl. drops of saline or *Rhodnius* haemolymph⁵. Saline containing 'Zectran' or amino-'Zectran' did not initiate secretion by isolated tubules, nor were tubules from insects paralysed with γ-hexachlorocyclohexane or allethrin altered in their normal response to secretory stimulants such as diuretic hormone⁵, serotonin⁵, or cyclic adenosine-3'-5'-monophosphate (unpublished results of S. H. P. M.). Addition to the saline drop of a whole mesothoracic ganglionic mass with its associated abdominal nerves intact did not stimulate secretion by isolated tubules, whether or not amino-'Zectran' was present. The mesothoracic ganglionic mass in *Rhodnius* consists of the mesothoracic and metathoracic ganglia fused with all the abdominal ganglia. These findings indicate that if secretion by the Malpighian tubules is stimulated on insecticide poisoning then an integration of processes in the living insect involving several steps is likely to be involved. A diuretic hormone is released into the haemolymph of *Rhodnius* after feeding⁶ and so we looked for diuretic activity in the haemolymph of insecticide-poisoned *Rhodnius*. Haemolymph taken from bugs at various times after topical application of 'Zectran' (200 µg/g) was tested for diuretic activity on Malpighian tubules isolated from untreated insects. Active samples induced the tubules to secrete at a high rate for a few minutes and the volume of secretion in 1 h was a measure of the amount of activity in the haemolymph sample assayed. The quantity of fluid secreted in 1 h by a tubule immersed in the drop of haemolymph was therefore taken as the measure of diuretic activity in the sample. After haemolymph had been taken from an insect, it was dissected so that the quantity of fluid in

the nearly spherical rectum could be estimated from a measurement of its diameter made with an ocular micrometer. The fluid accumulated after application of the insecticide could thus be determined and the rate of accumulation could be obtained from such measurements on many insects. The time of appearance of diuretic activity in the haemolymph and the rate of accumulation of rectal fluid were compared in a study involving more than forty insects (Fig. 1). Clearly there is a very good correlation between the rate of accumulation of fluid in the rectum and the level of a diuretic factor in the haemolymph. We therefore conclude that the appearance of fluid in the recta of insects poisoned with insecticides is due to the presence in the haemolymph of a factor which induces rapid secretion of fluid by the Malpighian tubules.

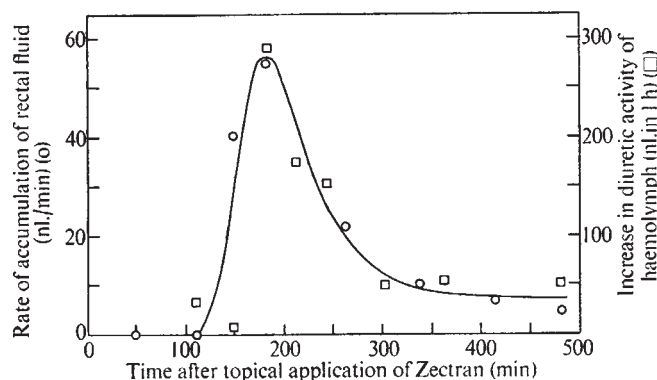


Fig. 1 Induction of diuretic activity in the haemolymph and of excretion in *Rhodnius* treated topically with 'Zectran' at 200 µg/g.

There is some evidence that the diuretic factor released on insecticide poisoning is the same as the diuretic hormone released when *Rhodnius* takes a blood meal. Secretion is initiated by insecticide chemicals which act primarily as nerve poisons and during the paralytic stage of poisoning. It is known that the diuretic hormone is released in fed *Rhodnius* from neurosecretory axons in the abdominal nerves close to their attachment to the mesothoracic ganglionic mass⁷. Ligature and surgical procedures originally used to localize the site of release of diuretic hormone when *Rhodnius* is fed^{6,7} were applied to insects poisoned with insecticide chemicals. Ligature experiments established that regions of the insect anterior to the mesothoracic ganglionic mass are not critical in insecticide-induced excretion, but that those posterior to this region are critical. Experiments involving surgery on the central nervous system showed that excretion following treatment with insecticide was not impaired in any of forty-five insects in which the ventral nerve cord was cut between the pro- and mesothoracic ganglia, but that in each of forty-two insects in which the cut was made just behind the mesothoracic ganglionic mass the insecticides no longer induced excretion. An average of 8.6 µl. of fluid accumulated in the rectum within 18 h in the former series, and only an average of 0.6 µl. in the latter series; an average of 0.4 µl. was found in unoperated and untreated insects. Significantly, the results were the same using DDT, γ-hexachlorocyclohexane, aldrin, dicrotophos, amino-'Zectran', allethrin or nicotine for poisoning. In each case, the mesothoracic ganglionic mass appears to be the site of insecticide-induced release of diuretic factor, strongly suggesting that the factor is the same as the diuretic hormone.

The diuretic activity present in homogenates of the mesothoracic ganglionic mass of *Rhodnius* can be distinguished from that of serotonin by selective bioassay because both compounds stimulate secretion by *Rhodnius* Malpighian tubules but only serotonin is of high potency in stimulating secretion by *Calli-*

phora salivary glands (unpublished results of S. H. P. M.). The insect nervous system is known to contain serotonin^{8,9}. Surprisingly the diuretic hormone found in the haemolymph after feeding or insecticide poisoning elicits a response in these selective assay systems intermediate between, and yet distinctly different from, that produced by either serotonin or breis of the mesothoracic ganglionic mass. Assay of several serotonin analogues on isolated tubules showed that they secrete rapidly only in the presence of compounds closely related to serotonin (unpublished results of S. H. P. M.). It is consistent with the available data to suggest that the diuretic hormone is an indolealkylamine or contains such a moiety in its structure. Such a substance could be bound in the neurosecretory cells as a Schiff base¹⁰ or in some other form such that feeding or insecticide-induced paralysis initiates its release for circulation in a free form. The information which provokes hormone release may be supplied by abdominal stretch receptors sensitive to vertical distension of the abdomen on feeding⁶ or by disruption in the intact reflex arc on insecticide poisoning.

These findings on the excretory physiology of insects are of potential importance in the use and design of insecticide chemicals. The rapid loss of a large volume of haemolymph by secretion of fluid into the rectum disrupts the critical water balance in the insect and may enhance the likelihood of the insect dying by desiccation. Furthermore, the composition of the remaining haemolymph probably differs greatly from that before insecticide poisoning, because the Malpighian tubules secrete a fluid which is in general extremely rich in potassium, high in phosphate and low in sodium and chloride ions¹¹. Not only is the ionic balance likely to change drastically, but the insecticide concentration in the haemolymph may increase as a result of the fluid loss and so enhance its disruptive action on the nervous system. Finally, these changes can be initiated by chemicals that paralyse the insect for only brief periods, because of their biodegradability or for other reasons, and it is therefore possible that insecticide chemicals that are nonpersistent in the environment could be used not as acutely lethal paralytic agents but as initiators of disadvantageous excretion.

This investigation was supported in part by grants to the University of California from the US Public Health Service, the Rockefeller Foundation and the Guggenheim Foundation. For advice and assistance, we thank M. J. Berridge, B. O. C. Gardiner, J. P. Heslop, and J. E. Treherne.

SIMON H. P. MADDRELL
JOHN E. CASIDA *

ARC Unit of Invertebrate Chemistry and Physiology,
Department of Zoology,
Downing Street,
Cambridge CB2 3EJ

Received September 1, 1970.

* Present address: Division of Entomology, University of California, Berkeley, California 94720.

- Spiller, D., thesis, Univ. Cambridge (1955).
- Jochum, F., *Höfchen-Briefe*, 9, 289 (1956).
- Roan, C. C., and Hopkins, T. L., *Ann. Rev. Entomol.*, 6, 333 (1961).
- Roberts, R. B., Miskus, R. P., Duckles, C. K., and Sakai, T. T., *J. Agric. Food Chem.*, 17, 107 (1969).
- Maddrell, S. H. P., *J. Exp. Biol.*, 51, 71 (1969).
- Maddrell, S. H. P., *J. Exp. Biol.*, 41, 459 (1964).
- Maddrell, S. H. P., *J. Exp. Biol.*, 45, 499 (1966).
- Berridge, M. J., and Patel, N. G., *Science*, 162, 462 (1968).
- Colhoun, E. H., in *Insects and Physiology* (edit. by Beament, J. W. L., and Treherne, J. E.), 201 (Oliver and Boyd, London, 1967).
- Alivisatos, S. G. A., Papaphillis, A. D., Ungar, F., and Seth, P. K., *Nature*, 226, 455 (1970).
- Berridge, M. J., in *Insects and Physiology* (edit. by Beament, J. W. L., and Treherne, J. E.), 329 (Oliver and Boyd, London, 1967).

Birth Order of Psychiatric Patients

Hare and Price¹ stated that in studies of birth order, schizophrenics should be compared with a control group because of anticipated birth-rate biases attributable to change in birth rate and family size. But their control group was limited to psychiatric patients with diagnoses other than schizophrenia. Such a sample may be expected to differ from the normal population in various respects, including birth order.

Further analysis of this data¹ indicates that their control group of non-schizophrenic patients differed from the normal population in birth order distribution. Table 1 shows the number of patients in the first, second, intermediate, penultimate and last ordinal positions from families of five or more children. The expected numbers are calculated assuming that in each family size, cases are equally distributed among the ordinal positions. There is a marked over-representation of last born cases, compared with each other position, including the adjacent penultimate position. The pattern is closely similar and statistically highly reliable for both sexes. Schizophrenics of both sexes have a similar pattern of over-representation of the last birth position (Table 2) which supports previous conclusions²; with the smaller number of cases, the χ^2 values are short of statistical significance, with the exception of the comparison between the last born and penultimate cases for males.

Table 1 Observed and Expected Number of Non-schizophrenic Psychiatric Patients in Each Designated Birth Order from Families of Five to Nine Children

Birth order	Observed	Males Expected	Difference	Observed	Females Expected	Difference
First	386	411.7	-25.7	502	531.9	-29.9
Second	374	411.7	-37.7	482	531.9	-49.9
Intermediate	862	881.0	-19.0	1,077	1,161.2	-84.2
Penultimate	383	411.7	-28.7	529	531.9	-2.9
Last	523	411.7	111.3	699	531.9	167.1

All five birth orders $\chi^2 = 37.56$; $df = 4$; $P < 0.001$. $\chi^2 = 64.98$; $df = 4$; $P < 0.001$.

Penultimate compared with last birth order $\chi^2 = 21.33$; $df = 1$; $P < 0.001$. $\chi^2 = 23.26$; $df = 1$; $P < 0.001$.

Table 2 Observed and Expected Number of Schizophrenic Patients in Each Designated Birth Order from Families of Five to Nine Children

Birth order	Observed	Males Expected	Difference	Observed	Females Expected	Difference
First	40	40.8	-0.8	34	45.3	-11.3
Second	40	40.8	-0.8	46	45.3	0.7
Intermediate	77	85.3	-8.3	104	98.7	5.3
Penultimate	36	40.8	-4.8	41	45.3	-4.3
Last	56	40.8	15.2	55	45.3	9.7

All five birth orders $\chi^2 = 7.07$; $df = 4$; $0.10 < P$. $\chi^2 = 5.60$; $df = 4$; $0.10 < P$.

Penultimate compared with last birth order $\chi^2 = 3.93$; $df = 1$; $P < 0.05$. $\chi^2 = 1.76$; $df = 1$; $0.10 < P$.

Hare and Price³⁻⁴ have clearly demonstrated that population changes can make the common assumption that births in the total population are equally distributed among all the ordinal positions inaccurate. For example, a trend towards smaller families in Great Britain has resulted in a decline in the number of large families. This tends to increase the proportion of people from the later birth orders of large families, because the higher birth rates in previous years than in subsequent years for such families result in a larger number of older than younger siblings. This does not, however, provide a satisfactory explanation for the birth order distributions of psychiatric patients from

families of five or more children (Table 1). The large difference between the number of penultimate and last born cases was not found for any other adjacent birth orders. The opposite trend occurred from the first to the second born. In contrast to these results, the birth order bias in the total population should apply in a constant, progressive fashion to each equally spaced ordinal position.

Other studies also indicate that the last born position can make the child more vulnerable to several different psychiatric illnesses. An illustration is the over-representation of last born cases, noted by Davis⁵ for a sample of homosexual males reported by Slater⁶. A similar effect, in several samples of male alcoholics⁷, has also been found in an unpublished study of 683 cases in the United States. The sample in Table 1 presumably included patients belonging to both of these categories; in particular some of the same homosexual patients⁶ were included, as they were from the same hospital during an overlapping span of years. These results for psychiatric patients contrast with other categories which contain an over-representation of first born cases. These include persons who have achieved intellectual distinction, including college students⁸, in particular those who subsequently develop hypertension⁹, as well as outstanding jet pilots¹⁰.

Research on birth order has been greatly aided by the fact that births in normal and stable circumstances can be expected to be equally distributed among the ordinal positions. Because of variations in birth rates at different times and places, biases based on birth rate changes can be minimized by using a population which includes a wide range of birth dates and localities, especially when pooling together data from different studies. In these conditions, persons who have gained distinction in various fields have shown highly consistent differences from the expected pattern of birth order distributions⁷. The proposed requirement of a control group for each sample¹ would severely retard research and might introduce greater errors than those remedied. An example is the specific over-representation of last born cases in their control group of non-schizophrenic psychiatric patients. Their data on schizophrenics (Table 2) agree with several earlier studies² to show that the later birth positions are over-represented in large families. They reached a contrary conclusion because of the erroneous assumption that the birth-order distribution of the non-schizophrenic control group, consisting entirely of psychiatric patients, resembled that of the total population.

H. B. III was supported by a US Public Health Service research scientist development award from the National Institute of Mental Health.

HERBERT BARRY, III

Department of Pharmacology,
University of Pittsburgh School of Pharmacy,
Pittsburgh,
Pennsylvania 15213

HERBERT BARRY, JUN.

Department of Psychiatry,
Massachusetts General Hospital,
Boston,
Massachusetts 02114

Received March 24, 1971.

¹ Hare, E. H., and Price, J. S., *Nature*, **228**, 1223 (1970).

² Barry, III, H., and Barry, jun., H., *Nature*, **223**, 752 (1969).

³ Hare, E. H., and Price, J. S., *Brit. J. Psychiat.*, **115**, 647 (1969).

⁴ Hare, E. H., and Price, J. S., *Brit. J. Psychiat.*, **116**, 409 (1970).

⁵ Davis, D. R., *Lancet*, **i**, 540 (1962).

⁶ Slater, E., *Lancet*, **i**, 69 (1962).

⁷ Barry, jun., H., Barry, III, H., and Blane, H. T., *Quart. J. Studies Alcohol.*, **30**, 408 (1969).

⁸ Altus, W. D., *Science*, **151**, 44 (1966).

⁹ Paffenbarger, jun., R. S., Thorne, M. C., and Wing, A. L., *Amer. J. Epidemiol.*, **88**, 25 (1968).

¹⁰ Reinhardt, R. F., *Amer. J. Psychiat.*, **127**, 732 (1970).

Retrieval of Information after Use of Marihuana

THERE is anecdotal and experimental evidence that human memory is adversely affected by smoking marihuana^{1,2} but it remains to be determined where in the memory system this impairment occurs. In particular, does marihuana interfere with the encoding or registration of information in the memory or with the retrieval of information once it has been stored? This study is a preliminary investigation of the effects of marihuana on the retrieval aspect of memory.

Adult men and women between 21 and 30 were invited to take part in an experiment dealing with memory. Volunteers were asked whether they had previously used marihuana and only those admitting to such usage were then asked whether they wished to participate in one of the conditions of the experiment, that was to be given marihuana. Those not admitting usage were never given this option.

The experiment^{3,4} involved the reading, to individual subjects, of fifteen lists of ten words each. Immediately after each ten word list, the subject was required to recall, in any order, as many of the words as he could remember; this tested immediate free recall (IFR). They were allowed 1 min for this, after which the experimenter began to read the next list.

The Mann-Whitney test indicated that the groups also did not differ on the DFR task or on the number of "old" words correctly recognized as being "old" (that is, the number of "hits"). Subjects given marihuana, however, reported significantly more "new" items as being "old" ("false alarms") than did controls ($U=15$, $P<0.01$). If the principles of signal detection theory⁷ are applied to the recognition task, it is possible to examine the ratio of "hits" to "false alarms" on the basis of the subject's true sensitivity (d') independent of any motivational factors or response biases which might have affected his discrimination between items, and it is also possible to determine how stringent a criterion (β) the subject uses in making his decision.

Analysis of these two indices indicates that not only does marihuana impair sensitivity or acuity in this type of discrimination task, it also tends to lower significantly the criterion on which he bases his decisions (Table 1). On the other hand, the scores from the Jackson⁵ achievement motivation scales did not differ significantly for the two groups, which suggests that although marihuana lowers the subject's criterion, he, or she, still wishes to do as well on the experimental tasks as the control subject who has not been given marihuana.

The non-significant results of the DFR test suggest that marihuana does not interfere with the retrieval of information

Table 1 Effects of Marihuana on Free Recall, Recognition and Achievement Motivation

Condition	Hits	False alarms	d'	β	IFR	DFR	Achievements
Control	63.5	8.7	1.068	2.560	100.6	13.9	11.1
Marihuana	77.7	38.9	0.667	1.209	91.9	15.2	10.4
	$U=31$	$U=15$	$U=1$	$U=13$	$U=8$	$U=36$	$U=41$
	n.s.	$P<0.01$	$P<0.03$	$P<0.01$	n.s.	n.s.	n.s.

After recall of the last list, experimental subjects smoked one marihuana cigarette (with an undetermined tetrahydrocannabinol content) for about 5 min. During the remainder of the interval before retesting, subjects were free to relax, read, or eat food that had been provided for them. Controls were treated exactly alike except that they were given no marihuana. Twenty-five minutes after the last list had been read to them, subjects were given 5 min to write out as many of the words in the previous lists as they could remember; this tested delayed free recall (DFR). The subject was then given a list of three hundred words containing the one hundred and fifty "old" words interspersed with the same number of "new" words, and asked to encircle all words which he felt had been on the lists previously read to him. After completing this task, the Jackson personality research form (BB)⁵ was administered to each subject to determine whether there were any differences between groups in their responses to the "achievement motivation" statements as a result of smoking marihuana. Jackson⁵ has defined achievement motivation as a willingness "to put forth effort to attain excellence". The purpose of this questionnaire was to find out whether subjects in the marihuana group were less motivated to do well on the recall and recognition tasks. As a result, any differences obtained would be attributable not so much to impairment of retrieval processes, but rather to the fact that subjects in the marihuana group are not willing to put forth the effort needed to perform well on the memory tasks.

Of the twenty-four volunteers, ten subjects per group were matched as closely as possible on the basis of their performance in the IFR test. A Mann-Whitney analysis⁶ indicated that the matching had resulted in no significant differences between groups on this initial, pre-treatment task. These mean scores, along with those from the DFR, recognition, and motivation tasks, are presented in Table 1.

already stored in the memory. This then suggests that any impairment in memory caused by marihuana occurs because information fails to become encoded in the memory stores and therefore is not available for retrieval. We are examining this possibility at present.

To refer to the recognition task, however, it is clear that marihuana renders the individual less sensitive in discrimination problems involving a delayed response task. The "hit" and "false alarm" rates indicate that the marihuana and control groups were separated only by the number of "new" items each was willing to accept as "old". In other words, subjects under the influence of marihuana were as capable of picking out "old" items from the lists as the controls, but were less inclined to reject "new" items. This increased willingness to accept, or decreased tendency to reject, is similarly reflected in the lower criterion scores of subjects under the influence of marihuana.

ERNEST L. ABEL

Department of Psychology,
University of Toronto

Received February 17, 1971.

¹ Abel, E. L., *Nature*, **227**, 1151 (1970).

² Tart, C. T., *Nature*, **226**, 701 (1970).

³ Cohen, R. L., *J. Verbal Learning and Verbal Behavior* (in the press).

⁴ Craik, F. I. M., *J. Verbal Learning and Verbal Behavior*, **9**, 143 (1970).

⁵ Jackson, D. N., *Personality Research Form* (Research Psychologists Press, Goshen, New York, 1966).

⁶ Siegel, S., *Nonparametric Statistics* (McGraw-Hill, New York, 1956).

⁷ Green, D. M., and Swets, J. A., *Signal Detection Theory and Psychophysics* (Wiley, New York, 1966).

Dream Recall and the Bias of Intellectual Ability

MANY people who gain high scores on conventional IQ tests are less successful on open ended tests that require mental fluency and imaginativeness¹⁻⁴. Such individuals have been called convergers, and those with the opposite bias, divergers³; convergers are more likely to specialize in the physical sciences, divergers in the arts^{3,5}. Open ended tests are themselves widely assumed to measure creativity, but the association with arts or science specialization suggests that this is an oversimplification.

It has been suggested that the strength of the converger-scientist in analytic reasoning and his relative weakness on open ended tests arise from a more general inhibition in dealing with the non-rational, and in particular the personal, emotionally charged aspects of life³. We tested this hypothesis by contrasting the ability of convergers and divergers to recall dreams. If inhibition is a general feature, convergers should recall fewer dreams than divergers, and their dream reports should be shorter and less eventful. If differences are slight, however, the convergers' relative weakness on open ended tests could reasonably be interpreted in terms of antipathy towards tests of an "unscientific" or unfamiliar kind.

Two matched groups of five convergers and five divergers were selected by the usual tests³ from a larger group of student volunteers. Each spent 5 nights in an EEG sleep laboratory and electroencephalograms, electroculograms and submental electromyograms were recorded. To avoid influence of the "first night effect" (ref. 6) on dream recall, subjects were woken only on the last 3 nights. The percentage of total sleep spent in rapid eye movement (REM) periods on the first 2 nights was used as an index of laboratory adaptation⁶; on night two, the two groups differed little in this respect (convergers 18.1%, divergers 20.5%).

Subjects were woken on nights three, four and five from all REM periods commenced after 0400 h by a burst of feedback on the intercommunication system. It is well known that dreaming occurs during REM periods⁷ and in these periods subjects are most likely to report the interruption of an immediate, vivid experience when waking has directly followed eye movements (REM-M waking)⁸. For this reason, subjects in our experiment were woken between 10 and 12 min after the start of each REM period, at a time when eye movements were observed. If no eye movements occurred during these two minutes, a REM-Q (quiescent) waking⁸ was made. Following a standardized interview procedure, subjects were asked: "Was anything going through your mind?". In the absence of recall, they were further asked to "See if anything comes back to you". After twenty seconds without recall, all interviews were ended by repeating that any content, however fragmentary, should be reported. Dream reports were taped and transcribed.

Table 1 shows that although convergers had almost as many REM periods as divergers, they were significantly less likely to recall dreams ($P < 0.005$). Convergers recalled dreams after only 65.0% of wakings, divergers did so after 95.2%; the norm for laboratory recall is about 80% to 85% (ref. 9). There were only two occasions on which a diverger was woken without recalling a dream; both cases involved the same subject, and took place on a night when he developed influenza.

The dreams which convergers recalled were also shorter

than those recalled by divergers ($P < 0.005$); the mean numbers of words used were 58 and 196 respectively. Convergers' dreams, too, involved fewer people and animals ($P < 0.01$), fewer interactions between the dreamer and other people ($P < 0.03$) and fewer aggressive interactions ($P < 0.01$). (The Hall and Van de Castle scale¹⁰ was used.)

The lower rate of dream recall among convergers can be explained in terms either of lower motivation or of lower incidence of vivid and therefore memorable dreams or of lower ability to recall. The first interpretation seems improbable, for subjects were paid volunteers, who believed that the aim of the research was to collect evidence of dreams; convergers expressed annoyance when they failed to provide this. The second also seems unlikely because profusion of eye movements is recognized as an index of dream vividness^{11,12}, and using this index, groups selected as non-recallers have been found to have dreams as vivid as¹³, or more vivid than¹⁴, those of recallers. The only subject with more than two REM-quiescent wakings was the diverger who was ill, and all his wakings lacked eye movements on the night of his recall failures. It is therefore probable that both convergers and divergers were experiencing vivid dream images immediately before the majority of wakings.

The most likely interpretation seems to be the third—that the two groups differ in their ability to recall. In eight of the fourteen instances of non-recall among convergers, subjects asserted that they had been dreaming but could not recall the dream content. The dreams they did recall were shorter, but not because convergers are necessarily less fluent than divergers; convergence or divergence is a measure of bias, and so a few convergers gain such high IQ scores that their open ended scores are higher, rather than lower, than those of some divergers. Two such convergers were selected for this experiment and they reported dreams more briefly than did the two divergers whose scores on the open ended tests they exceeded.

These findings support Hudson's contention that the differences in intellectual bias between convergers and divergers reflect a difference in temperament. There appears to be a general inhibition or repression among convergers about the emotional and non-rational, which reinforces their capacity for "logical construction" at the expense of "combinatory play". These terms were coined by Einstein, who considered combinatory play a central feature of his productive thought¹⁵. The distinction is also reminiscent of Freud's differentiation between ordered "secondary process" and unstructured "primary process" thinking¹⁶. Both lack of creativity and failure to recall dreams were attributed by Freud to the repression of primary process thought.

I thank Professor Liam Hudson and Dr Ian Oswald for their help.

M. D. AUSTIN

Department of Educational Sciences,
University of Edinburgh

Received December 21, 1970.

¹ Getzels, J. W., and Jackson, P. W., *Creativity and Intelligence* (Wiley, New York, 1962).

² Wallach, M. A., and Kogan, N., *Modes of Thinking in Young Children* (Holt, Rinehart and Winston, New York, 1965).

³ Hudson, L., *Contrary Imaginations* (Methuen, London, 1966).

⁴ Cropley, A., and Maslany, G., *Brit. J. Psychol.*, **60**, 395 (1969).

⁵ Povey, R. M., *Brit. J. Psychol.*, **61**, 55 (1970).

⁶ Rechtschaffen, A., and Verdone, P., *Percep. Motor Skills*, **19**, 947 (1964).

⁷ Aserinsky, E., and Kleitman, N., *Science*, **118**, 273 (1953).

⁸ Molinari, S., and Foulkes, D., *Percep. Motor Skills*, **29**, 343 (1969).

⁹ Dement, W. C., in *New Directions in Psychology II* (edit. by Edwards, W., Lindmann, H., and Phillips, L.) (Holt, Rinehart and Winston, New York, 1965).

¹⁰ Hall, C., and Van de Castle, R. L., *The Content Analysis of Dreams* (Meredith, New York, 1964).

¹¹ Dement, W. C., and Wolpert, E. A., *J. Exp. Psychol.*, **55**, 543 (1958).

Table 1 Number of Dreams Recalled after Waking from REM Sleep by Convergers ($n=5$) and Divergers ($n=5$)

	Convergers	Divergers
REM period wakings *	40	42
Dreams recalled	26	40
Recall as percentage of wakings	65.0	95.2

* REM-M and REM-Q.

- ¹² Berger, R. J., and Oswald, I., *Science*, **137**, 601 (1962).
¹³ Lewis, H. B., Goodenough, D. R., Shapiro, A., and Sleser, I., *J. Abnorm. Psychol.*, **71**, 52 (1966).
¹⁴ Antrobus, J. S., Dement, W. C., and Fisher, C., *J. Abnorm. Soc. Psychol.*, **69**, 341 (1964).
¹⁵ Hadamard, J., *The Psychology of Invention in the Mathematical Field* (Dover, New York, 1954).
¹⁶ Freud, S., *The Interpretation of Dreams* (Hogarth Press, London, 1953).

Basic Rectangle of the Mandible in the Hominoidae

KINZEY's letter to *Nature*¹ calls for a reply. I do not criticize him, of course, for preferring to translate my visual "basic rectangle" into a standard type of index, which he calls "the BRM index", but it is his attempt to utilize this index to refute my claim that *Kenyapithecus africanus* is taxonomically within the family Hominidae that requires critical comment.

So far as I am aware, neither I nor anybody else has attempted to use evidence based on the basic rectangle of the mandible, for taxonomic purposes, beyond the limits of the superfamily—the Hominoidae. Kinzey's attempt therefore to introduce evidence from representatives of other families of the order primates, even including the Tarsoidea and Ceboidae, is irrelevant to the argument about the taxonomic value of the basic rectangle in studies of the Hominoidae.

Kinzey's communication seems to be special pleading. He entitled his Fig. 1 as follows, "Basic rectangle of the mandible in representative genera of living primates. . . . The BRM index for each is given in parenthesis". This wording suggests to the reader that the basic rectangles and the indices quoted with them are "representative" of the various genera which he illustrates. But on the next page he states that what he lists in Fig. 1B as "*Homo sapiens*, negro (116)" is not representative but is "the extreme case".

Similarly, when his Fig. 1A, which he entitles "*Homo sapiens*, white (77)" is compared with the information given elsewhere in his letter, the index of 77 which he quotes as being that of what he calls "*Homo sapiens*, white" is in the first place based only on what he describes as "Twelve American white mandibles" and, moreover, that these twelve yielded an average index of 86.7. The example which he illustrates with an index of 77 in Fig. 1A is therefore also not representative.

At this point it is perhaps pertinent to ask what Kinzey means by "*Homo sapiens*, white" and "*Homo sapiens*, negro". Is he seriously suggesting that he believes that either the white skinned or the dark skinned populations of the United States, or of the world, are so homogeneous as to be capable of being considered valid anthropological entities?

Kinzey goes on to say that the indices which he has worked out confirm my statement that the shape of the mandibular rectangle "in living man differs clearly from that of living pongids"; but he then proceeds to adduce a great deal of special pleading in an attempt to demonstrate that this is not, in his personal view, true of the fossil representatives of the two families. In his Fig. 2, for example, he shows two excessively warped and abnormal fossil hominid mandibles, those of *Australopithecus robustus* (No. SK 23) and of *Homo sp.* (No. SK 15)* which are both from the Swartkrans site in the Transvaal, and uses them as supposed evidence that some australopithecines fall "within the estimated population range for living pongids". The evidence which he derives from these two specimens is meaningless, because the bone of both specimens is known to be badly warped and this has been stated in print many times. It must also be pointed out that careful reconstructions of what these mandibles must have looked like before they were warped have been published.

Kinzey does state that he realizes that some of his measurements "may be based on warped specimens" and, moreover, his

measurements are chiefly based on "casts or photographs", not on the original specimens. Yet he does not hesitate to use such evidence to support his theory, without apparently checking with the institutions where the originals are kept.

Later in his letter he says: "the similarity in the BRM index of the two Rusinga mandibles in Fig. 2 supports the suggestion . . . that mandible No. 394 (of 1967, the *Kenyapithecus* mandible) is most similar in its overall morphology to *Dryopithecus* (*Proconsul*) *africanus*". He then goes on, "If *Kenyapithecus africanus* is linked clearly with the family Hominidae on the basis of the shape of the basic rectangle, then *Proconsul africanus* must be also". Just as in the case of treating *Australopithecus robustus* as lying within the range of the indices of the Pongidae on the basis of measurements taken from a photograph of the warped specimen (SK 23), so the statement about the supposed similarity of the mandible of *Proconsul africanus* (No. R 1947 375) to *Kenyapithecus africanus* (No. R 1967 384) is misleading.

Le Gros Clark and I² made it clear that in the case of this particular *Proconsul africanus* specimen (No. R 1947 375), the enamel of most of the crowns of the teeth was badly damaged and broken away, the incisors and canines were all broken off and the anterior region of the alveolar margin was damaged. Unfortunately, Kinzey does not state the BRM index which he obtained by measuring our photograph of this specimen, but in his Fig. 2 he positions it at a point which gives it an approximate index of 122. This places it, he says, "almost within the estimated population range of American negro". Actually, when I re-examined this *Proconsul* specimen, it became clear that there is at least 1.5 mm of enamel missing at the back of the third molars, and approximately 1 mm missing at the front of the alveolar margin. Estimated as accurately as possible from the original, the length of the basic rectangle of this specimen is 51 mm and the width 39 mm which yields a BRM index of 130.9 compared with Kinzey's index of 122 based on his measurements of the photograph. This places this *Proconsul* specimen well within his extended pongid range and very much further away from the position of *Kenyapithecus africanus*, which lies within the range of *Homo sapiens*, as shown by Kinzey's Fig. 2.

I assume that Kinzey accepts the theory of evolution and believes therefore that the further back the families Hominidae and Pongidae are traced, the more likely they are to be morphologically similar, whereas their present-day representatives are likely to have become more and more divergent as each specialized away from the common stock. It is therefore to be expected, as is the case, that the pongid *Proconsul africanus* and the hominid *Kenyapithecus africanus*, both from Lower Miocene deposits of Rusinga Island, would be morphologically rather more similar to each other than present-day man is to living great apes.

I never attempted to use the single factor of the shape of the basic mandibular rectangle of *Kenyapithecus africanus* when I decided to include it, taxonomically, within the Hominidae. I only used the evidence from the mandibular rectangle as one of a large number of different characteristics to be seen in the specimens, every one of which pointed in the same definite direction.

I suggest that when Kinzey's statements are adjusted in relation to the basic facts which I have outlined, his evidence supports rather than disproves my view that *Kenyapithecus africanus* is a primitive member of the family Hominidae.

L. S. B. LEAKEY

Centre for Prehistory and Palaeontology,
PO Box 30239,
Nairobi

Received January 5, 1971.

¹ Kinzey, W. G., *Nature*, **228**, 289 (1970).

² Le Gros Clark, W. E., and Leakey, L. S. B., *Fossil Mammals of Africa*, No. 1 (British Museum (Natural History), London, 1951).

* *Telanthropus capensis* has now been shown to be *Homo*. The specific name *capensis* is invalid for *Homo*, having been used before.

BOOK REVIEWS

Who will Call the Tune?

The Dilemma of Accountability in Modern Government: Independence versus Control. Edited by Bruce L. R. Smith and D. C. Hague. Pp. xviii + 391. (Macmillan: London, February 1971.) £4.50.

THIS is a pioneer book, important enough to have been written in simpler English for a wider audience. It consists of two lengthy comments by Professor Douglas Hague, of Manchester University, and Professor Bruce Smith, of Columbia University, and of the working papers of a Ditchley conference, held in March 1969, to consider "a new phenomenon in the art of government"—the problems of accountability and independence.

Alan Pifer, president of the Carnegie Corporation of New York, had for some time been concerned with the growing practice of federal government to devolve responsibility to private organizations on a contractual basis. But certain questions remained unanswered: what were the public benefits of this practice; what did it contribute to the financial viability of private institutions; above all, what were the anatomical characteristics of the new quasi-non-governmental sector?

From England, David Howell, MP, now parliamentary secretary for the Civil Service, drew his attention to a more basic aspect: how could government respond effectively to the speed of change, and remain accountable to the electorate? That was in 1967. There followed the setting up of parallel study groups in the US and the UK; these met at Ditchley, of which get-together this book is the product. A second book based on further work is promised. This is necessary, because what is missing is a coordinating principle, a central hypothesis which will see the phenomena it deals with as parts of a whole.

For Professor Smith, so extensive is the use of "administration by contract", especially in the US, that the modern state may be described also as "the contract state". British interest was not as broad. It was concerned chiefly with how to combine accountability with independence in the nationalized industries. Both groups began to put more emphasis on the quasi-non-governmental organization (Qngo), and less on contracting with the private sector.

For the British participants, says Professor Hague, there came a revelation. Mrs Fanny Mitchell, under the direction of Professor W. J. Mackenzie, of Glasgow, has drawn up a list of more than 700

Qngos in Britain. They include the Law Commission, Bank of England, Regional Hospital Boards, Gas Council, General Medical Council, BBC and the like. Classification is not easy.

The Qngos receive an impressive sum of money annually from Government. From the 1969/70 estimates, £350 millions for grants; a similar sum for grants-in-aid (which means money left over at the end of the year does not have to be returned to the Treasury); and £65 millions for research and development. It is difficult to separate out exactly the total of monies going to the Qngos, but clearly the sum involved is large.

Why have the Qngos come into existence? Six reasons are suggested: to protect certain activities from political interference; to escape the weaknesses of bureaucratic rule by government departments; to make sure the right talent is engaged on the right tasks; to spread power by having more centres of responsibility; to provide an administrative "back double" to get things done outside the existing structure; to beat the "too-many-civil-servants" criticism.

Four kinds of accountability are distinguished: programme, process, fiscal, and social. This last was construed as responsibility to society. Sir Brian Flowers, chairman of the Science Research Council, gave as example the question of whether SRC scientific judgments were good. This was a sort of social accountability. "The scientific community, as the representatives of the public, was asking whether the judgments made were the right ones." I regard this as a most unsatisfactory example. The scientific community does not represent the public. Neither Sir Brian nor his colleagues—worthy though they are—are elected: they are appointed. They represent an establishment, or a special pressure group.

This links up with the question of independence. The Ditchley conference feeling was to see independence as a means to an end: Qngos would perform better if they had greater independence. Sir Brian suggested that Government departments would be incompetent in running research laboratories or research organizations directly. This is an interesting statement in view of the turmoil now on in Britain in an attempt to redefine the role of research councils, and the laboratories they control. In fact, many Qngos—under prodding by Government—are diversifying most successfully the laboratories they control.

Now that nationalized industries are

being pushed back, in part, into the private sector, the problems of accountability and independence are highly important. The criteria to judge the performance of Qngos need clear definition, especially as Ministers seem to differ in the extent to which they believe they may exert pressures. Attention has been drawn (see Maurice Corina in *The Times*, April 26, 1971) to the contradictory aspects, for instance, of two statements made at the October 1970 annual Conservative Party conference. Mr John Davies, Secretary of State for Trade and Industry, promised the "disengagement" of nationalized industries from excessive Governmental control; Mr Chris Chataway outlined his firm handling of the Post Office Corporation. Mr Corina concludes: "The Post Office can be faulted on many issues. But a fair share of responsibility must be allocated to the continuing confusion between the aims of Ministerial and Parliamentary control and the pursuit of commercial objectives."

I have an unhappy feeling that a Qngo is really an attempt to remove a problem that should concern Parliament directly from its context, to make it not a democratic construct, but to give it a reality in its own right: logically, then, Parliamentary democracy is required to adjust itself to that reality. What develops—to borrow a phrase from Thomas Mann—is "power-protected intimacy". This is not good for democratic behaviour. A Qngo is then rather like the psychiatrist defined as a doctor who hates the sight of blood.

It's a pity that neither the British nor the American study groups contain a representative of the civil service unions. Also, I'd like to see a statement on the problem of the Qngo in a developing country.

MAURICE GOLDSMITH

Early Cultivation

The First European Agriculture: a Study of the Osteological and Botanical Evidence until Two Thousand BC. By Jacqueline Murray. Pp. vii + 380. (Edinburgh University: Edinburgh, January 1971.) £3.00.

IN recent decades it has become standard archaeological practice to treat osteological material with the same care that is devoted to artefacts. Botanical material, though less obvious to the excavator and sometimes needing special sampling, is

also increasingly being given similar attention. As the number of sites excavated has increased, so the record of biological material has accumulated until it has reached quite formidable proportions. Whereas biologists have to some extent used such records for their own purposes (for example, Godwin's *History of the British Flora*), there have been few attempts to draw conclusions about how man learned to use and breed plants and animals for his own purposes. In this book, Dr Murray has attempted to trace the pattern of development of agricultural practices as they spread across Europe from their origins in the Near East. She draws on some 550 references, comprising a bibliography of 30 pages. Much of this material has been extracted in the form of histograms and tables which together make up nearly two-thirds of the thickness of the book. The actual text only amounts to 111 pages.

It is difficult to decide for whom this book has been written. There is very little digestion of the catalogue of facts presented, so the book cannot be aimed at the student, either in the archaeological or biological field. Is it therefore directed at the specialist as a book of reference? If so, this purpose is destroyed by the lack of an index of any sort. Even if an author fails to provide an index to a turgid text such as this, the publisher is failing in his duty if he does not insist on one.

Who, then, will profit by this book? It is indeed difficult to say. For the archaeologist there is surely much more to be deduced than has been done here. On the other hand, any biologist lacking considerable knowledge of European archaeology will be unable to evaluate the material because it is presented throughout in an archaeological context and with corresponding technicalities. No maps are provided to show the location of the many sites named, and no key is given to the cultures referred to, which cover a wide range of space and time. This is no doubt familiar to the specialist in the Neolithic; perhaps the book is really written for him.

The biologist will find himself questioning the uncritical presentation of some of the biological material. Is it really justifiable to refer to excavated skeletal material by 4-nomial Latin names? And is the extension of the description "turbary" to bones from far outside its original Swiss context really meaningful in any way? Even if the answers are affirmative, these points need discussion. The botany of the cereals, too, as presented here is in places out-of-date both as to nomenclature and genetics.

In spite of the scale of the literature referred to, the subject, as indicated by the title of the book, is still incompletely covered. There is, for instance, an extensive literature on pollen analysis touching on this theme, but the few examples selected were only marginally relevant.

It would need a botanist to assess this literature, but until this is done it cannot be said that all available material has been brought together.

Probably the great service that Murray has done is to collate the literature—much of it beyond the net of the average botanist or zoologist—so that agricultural specialists could now use it for the compilation of definitive works on the early developments of plant and animal husbandry. Murray has shown some of the broad lines which could be followed and it is clear that if this is not attempted soon, the amount of information to be incorporated will be almost unmanageable. The subject needs such studies to show where it has got to and to direct future specialization along lines which at present are less than adequately covered.

G. W. DIMBLEBY

In Pacific Waters

Marine Atlas of the Pacific Coastal Waters of South America. By Merritt R. Stevenson, Oscar G. Guillen and Jose Santoro de Ycaza. Pp. 23+charts. (University of California: Berkeley and Los Angeles, March 1971.) £11.90.

THE normal oceanographic conditions and climate of the west coast of South America are determined largely by the upwelling of very cold water between the slow-moving northward Peru current and the coast. The Peru current starts to turn west a few degrees south of the equator to join the south equatorial current, leaving a sensitive area in which a change in the balance of the forces producing upwelling and those bringing warmer water to the coast may lead to a rapid rise in temperature. The consequences to life in the sea are occasionally disastrous and their effects sometimes extend as far as 15° S.

Limited understanding of the basic factors has long been recognized as a serious challenge to marine science and the new atlas is based on a systematic study conducted by the Inter-American Tropical Tuna Commission with the help of marine laboratories in Columbia, Ecuador, Chile and Peru. The brief introductory text is printed in both English and Spanish. Part 1 deals with the sources and treatment of the data, and Part 2 with seasonal variations in the coastal region. The maps show station positions, surface distributions of temperature, salinity, density and oxygen content, observed winds, and surface currents inferred from the 0 to 200 m density distributions, at 3-monthly intervals from February–March 1964 to February–March 1966. The surface charts are generally supported by sections showing the vertical distributions out to several hundred miles from the

coast and mostly to a depth of 1,200 m. Near the equator a longer section was studied—from the coast to the Galapagos Islands.

Like earlier workers, the authors recognize a sharp boundary region, usually called the Intertropical Convergence Zone, between the poorly saline waters of the doldrum belt and Panama bight and the more saline water of the Peru current. They show that the boundary zone follows the Sun, migrating northwards in the northern summer and south in the southern summer, with a general tendency to bend southwards near the coast. They do not attempt a comprehensive, general explanation in terms of the prevailing wind systems, for which we must still learn much more, but they provide evidence of local interactions between the winds and currents, especially near the coast, and supplement their study of the effects of local warming and cooling, rainfall and evaporation, with a fair amount of information about the inflow of water from adjacent areas. They thus provide much new material, particularly in the rather little known equatorial region, to assist further study.

The maps are handsomely and carefully produced. They should be specially useful in the further study of animal distributions.

G. E. R. DEACON

From the Lunar Surface

The Lunar Rocks. By Brian Mason and William G. Melson. Pp. vi+179. (Wiley-Interscience: New York and London, January 1971.) £4.20.

SELENOGRAPHERS who have kept in touch with the results of the successful Luna, Ranger and Surveyor programmes of lunar exploration will not find much new in *The Lunar Rocks*. Nor will those who have been concerned with the findings of selenologists who have studied rocks and soil brought back from the Apollo 11, 12 and Luna 16 expeditions. This is not to say, however, that the book is not an excellent summary of information on the Moon's surface prior to the Apollo and Luna landings, and more especially of lunar mineralogy, petrology and geochemistry resulting from recent investigations of Apollo 11 rocks. The Second Lunar Science Conference held in January this year came too late for the authors to do much in the way of comparing and contrasting Apollo 11 and 12 rocks, but they have made good use of the LSPET report *Preliminary Examination of Lunar Samples from Apollo 12* (*Science*, 167, 1325; 1970). In addition, they have drawn on their own and their colleagues' first hand studies of Apollo 11 and 12 samples, and the senior author has effectively utilized his extensive knowledge of geochemistry and more particularly of

meteorites. The result is a concise and factual account of lunar rocks written so as to be of value to a wide spectrum of scientists as well as students and laymen.

Following the introductory chapter on selenography prior to Apollo and a relatively short one on the Apollo programme, the authors give appreciable—though somewhat uncritical—attention chiefly to the mineralogy of Apollo 11 rocks. They note in particular the similarity with terrestrial basalts, but rightly draw attention to differences such as the absence of ferric iron and the presence of native iron and minerals such as troilite, armalcolite and pyrox-ferroite as indicative of the low partial pressure of oxygen during crystallization.

The next two chapters deal with lunar petrology, again with the chief emphasis on Apollo 11 rocks. Most important features of the igneous rocks, microbreccias and fines are ably described and some comparisons made with Apollo 12 samples. The controversial question of remanent magnetism and to what extent thermal remanent magnetism has been modified by later shock induced magnetism is discussed. The authors express hope, tinged with doubt, that oriented samples from bedrock exposures may solve the problem of the Moon's past magnetic field.

In the next chapter petrographic comparisons are made with terrestrial rocks, meteorites and tektites. The authors emphasize the similarities between lunar and terrestrial basalts as well as differences such as the presence of rare minerals not known on Earth. They are sceptical about the possibility of material getting into Earth-crossing orbit after being ejected by meteorites or comets striking the Moon. But, assuming that it could, they see no evidence of chondrites being derived from the Moon, though there is a better case for calcium-rich achondrites. Similarly, they see little evidence so far from lunar samples to indicate that tektites are of lunar origin with the possible exception of one Apollo 12 specimen.

The composition of lunar rocks is dealt with in an important chapter on lunar geochemistry (39 pages). Elemental abundances are given and compared with the diabase W-1 (which for many years has been used as a geochemical standard), eucrites, and carbonaceous chondrites. Here the authors' critical judgment is particularly effective and the result is not only an excellent tabular display of data, but a discussion, element by element, of the analytical methods of determination and of the significance of different elemental concentrations. Particular attention is given to patterns of elemental enrichment and depletion as compared with terrestrial rocks, meteorites and possible cosmic abundances.

The final chapter considers the implications for lunar history of the most significant facts so far established by the Apollo

missions. It deals with the origin of the maria and other surface features, with the internal structure of the Moon and the nature of the mascons, and with the four principal hypotheses currently in vogue for the origin of the Moon. Of these, favour is given to the Öpik-Ringwood hypothesis which, briefly, suggests that the Moon was formed as a result of the coalescence of a ring of planetesimals that once surrounded the Earth following the disruption and subsequent cooling of a massive primitive atmosphere.

The book has a list of about 150 selected references and an author/subject index. It is well printed and illustrated and suffers little from being produced in a relatively short period of time. It is highly recommended to all interested in lunar rocks and in what the authors describe as having been "a unique scientific adventure, and an intellectual challenge of the first magnitude" to many scientists of different disciplines. S. H. U. BOWIE

Ordering and Stacking

Ordered Alloys: Structural Applications and Physical Metallurgy. Edited by Bernard H. Kear, Chester T. Sims, Norman S. Stoloff and Jack H. Westbrook. (Third Bolton Landing Conference, Lake George, New York, September 1969.) Pp. 580. (Claitor: Baton Rouge, 1970.)

It is unfortunate that the proceedings of this conference have taken so long to be published. The field covered continues to be an active area of research and certainly parts of this book are now somewhat dated. Also, as invariably happens, many of the papers contain material which was published elsewhere before the conference, or has since been published in a revised form. These criticisms apart, there is no doubt that this well produced and very well edited book will become a standard reference for research workers in the field. It may well also prove useful to advanced undergraduate students in materials science and related fields who need a source of critical articles for a project involving a literature survey in this general area.

The conference attempted to cover an extremely wide field, from the theory of order/disorder transformations to the engineering applications of materials containing ordered precipitate particles. The articles in the proceedings again have this same scope and it might have been expected that the volume would seem disjointed and the individual papers unrelated. All in all, this has not happened; the editors are to be congratulated on subsectioning the proceedings into well related groups of papers and a sometimes tenuous thread between the sections has been main-

tained. This thread is much easier to follow thanks to an excellent initial review by one of the editors (J. H. Westbrook) which gives an admirably brief survey of the whole field.

After this initial review, the first section consists of a series of papers on the theory of order and phase stability. These papers chiefly consider relatively conventional models of ordering, but there is a clear contribution in the attempts that have been made to refine the various models and present more quantitative interpretations of the property changes on ordering. The next section has papers on stacking and stacking faults. Many of the ideas presented here are taken up again in the papers more concerned with applications in the latter parts of the proceedings. The following section considers the mechanism and kinetics of ordering; much of the material here is the relatively conventional interpretation of kinetic experiments, but the simulation studies by Beeler using a computer indicate a relatively new departure. The next two sections cover the mechanical properties of, first, single phase intermetallics, and then multiphase alloys. A considerable number of these papers make important contributions to the theory of hardening due to the presence of ordered particles. The final section deals with engineering applications, covering fracture creep, fatigue and so on.

The continuing interest in this area suggests that it would be useful to have a repeat of this type of conference every few years.

B. RALPH

Plants and Parasites

Physiological Plant Pathology: An International Journal of Experimental Plant Pathology. Edited by T. F. Preece and B. J. Deverall. (Published quarterly by Academic: London and New York, 1971.) £7.00 annually.

INTEREST in the relationship between plant hosts and their parasites has increased greatly since 1920 when Professor W. Brown, FRS, began his distinguished researches on this subject at the Imperial College of Science and Technology, London. The new specialized journal, *Physiological Plant Pathology*, offers an outlet for papers on all aspects of the plant host/parasite relationship and can be expected to bring together much of the work which would otherwise be scattered in a number of more or less suitable journals.

The editors have wisely laid down fairly precise limits on the subject matter to be included in the new journal. Nevertheless the first number shows that they have not interpreted their ruling in any narrow sense but have accepted papers ranging

widely over the physiology of plant disease. Among the topics dealt with are the ultrastructural changes produced by disease, the biochemistry of phytoalexins and pectic enzymes, and factors influencing virus multiplication in the diseased plant, all of obvious interest to the physiological plant pathologist. Of less immediately obvious relevancy is a paper offering a scheme for the mechanism for the plasticizing of plant cell walls. That this paper is of interest to all plant physiologists and not only to plant pathologists indicates that the new journal should not be dismissed by botanists as of narrow specialist interest only. The journal's appeal and usefulness will be widely appreciated not only by those for whom it specifically caters but by plant physiologists, mycologists, plant virologists, biochemists and many others.

The authors are drawn from six different countries indicating that the editors intend to make this a truly international journal.

The standard of the articles in this first number is high, the journal is clearly printed on good quality paper and both line diagrams and photographs are satisfactorily reproduced. If these high standards are maintained (and the list of members of the editorial board guarantees that this will be so) the journal will be a welcome addition to biological periodicals. **LILIAN E. HAWKER**

Detoxicant Enzymes

Amine Oxidases and Methods for their Study. By R. Kapeller-Adler. Pp. xi + 319. (Wiley-Interscience: London and New York, December 1970.) £9.25.

SINCE the discovery of the enzyme monoamine oxidase by Hare in 1928, a bewildering array of amine oxidases has been described. This book surveys the whole of this literature, and provides a valuable guide for enzymologists and others with an interest in this group of enzymes.

After a somewhat lengthy introduction which reviews current theories of enzyme action, the author outlines the classification scheme of amine oxidases and proceeds to describe in detail the biochemical properties, cofactors, inhibitors, reaction mechanisms and biological significance of individual enzymes. A large section is devoted to classical monoamine oxidase, an intracellular insoluble flavoprotein found in many vertebrate and invertebrate tissues. Monoamine oxidase has a fairly well defined and important biological role in the detoxication of a variety of foreign and endogenous amines, such as the catecholamines and 5-hydroxytryptamine in animal tissues. Monoamine oxidase inhibitors include various drugs used in the treatment of depression and angina pectoris in man, and these

various inhibitors and their mode of action and pharmacology are fully described. Numerous other amine oxidases are also described, including diamine oxidase (histaminase), mescaline oxidase, extracellular amine oxidases of plasma, and plant and bacterial amine and polyamine oxidases. Unfortunately most of these enzymes do not yet have clearly defined biological roles; in many cases the natural substrates are not even known.

The second half of the book is devoted to a full description with practical details of laboratory methods used for the assay and for the isolation and purification of each individual enzyme. This very comprehensive collection of techniques will undoubtedly be of considerable value to researchers in this field. More critical comment from the author would have been welcome, however, in this and in other chapters of the book. The reader might like to know, for example, which of the eleven methods described for the assay of monoamine oxidase would best fit his requirements. There is a tendency to quote the conclusions of others too frequently; I would have preferred to see more critical analysis and clearer summaries of the author's own interpretations.

The bibliography with more than one thousand references is on a heroic scale, but its practical usefulness is limited by the non-alphabetical listing of items without titles. Nevertheless, this volume should certainly prove valuable as a reference work for students. It also provides a useful laboratory reference of techniques for biochemists interested in this large and often confusing group of enzymes. **L. L. IVERSEN**

Metabolism and Cancer

Homologies in Enzymes and Metabolic Pathways; and Metabolic Alterations in Cancer. Edited by W. J. Whelan and J. Schultz. (Proceedings of the Miami Winter Symposia, January 1970.) Pp. ix + 529. (North-Holland: Amsterdam and London, 1970.) Hfl. 90; £10.50; \$25.

THIS book contains the full papers presented to the Miami Winter Symposia during January 19–23, 1970, and is the first of a continuing series under the title "Miami Winter Symposia". Associated with the symposia is a lecture named in honour of the distinguished biochemist, Professor Feodor Lynen. The first Lynen lecture, given by Dr George Wald and entitled "Vision and the Mansions of Life", is reproduced in this volume.

The book comprises the first two volumes in the series: one called "Homologies in Enzymes and Metabolic Pathways", and the second called "Metabolic Alterations in Cancer". These volumes will appeal to the same general public,

but will not have identical audiences.

The first volume on evolutionary homology in enzymes includes a number of very thoughtfully compiled papers. Several authors have been working and thinking about their problems for many years; these articles show the fruit of their effort, and will bear rereading over a number of years. Some other articles are of interest as research reports.

The second volume on metabolic alterations in cancer includes five articles on immunochemistry. There are too many diverse subjects dealt with in this book to permit detailed consideration of each of them. By example, only, I shall comment on the excellent review by Weinhouse of his work on Morris hepatomas. The competition between glycolytic and respiratory formation of ATP, discussed as long ago as 1941 by Feodor Lynen and M. J. Johnson, seems to me to be of special significance in this context, although its physiological significance is by no means clear. The inverse relationship between tissue NAD levels, NAD pyrophosphorylase levels and tissue growth rate observed and emphasized by R. K. Morton is not discussed by Weinhouse, but seems very relevant. The absolute level of NAD may be involved in determining the pattern of metabolism, and the patterns observed by Weinhouse may be unrelated to the cancerous state of the cell, but may be a function of their growth rate. To provide a more profound understanding of the nature of the growth processes we need to unify our understanding of growth rate, levels of NAD and ATP, their rates of synthesis and degradation, and the patterns of energy metabolism associated with different growth rates.

These volumes eloquently demonstrate the development of biochemistry. At an early time we had a naive, integrated view of biological phenomena. During the last half century biochemists have analysed a number of biological processes in some detail and we now have a reasonable picture of the component processes of a living cell. Biological science is now entering the third stage; during this period we will evolve an integrated understanding of biological phenomena based on a vast, detailed empirical knowledge. This integrated view will form the starting point for a further progression in our knowledge through the same stages.

I found these two volumes enjoyable reading; they are of such lasting value that I shall strongly recommend them to students. The editors are to be congratulated on achieving relatively rapid publication. The wisdom of publishing symposia proceedings is still uncertain, and equal care will be needed to ensure useful subsequent volumes. Readers will look forward to these, hoping that they will be thoughtful and stimulating as well as providing brief critical reviews of recent new knowledge. **SYDNEY SHALL**

CORRESPONDENCE

Indian Brain Drain

SIR,—Much has been written about the brain drain from the developing (and, lately, from the developed countries). As an Indian with a PhD in chemistry who has immigrated to the USA, I have a vested interest in this problem. I am sure that my feelings are shared by many like me.

As a product of the liberal arts/natural science type of education, I have been the subject of a conditioning process which attempts to instil a belief in the innate desirability of the acquisition of academic goals and ideals of elders and teachers. If this conditioning is unsuccessful, one is left with a choice between frustration and dropping out—that is, immigration—though the two courses are not equivalent. Under such circumstances, I do not think that any approbation or censure should be attached to an individual's choice of action, as Dr Parthasarathi suggests (*Nature*, 230, 87; 1971).

The planning and execution of official policies in the developing countries are necessarily of profound importance. In India, for example, the vast resources invested in education have created mainly a local elite class, the *babus*, who fill the ranks of the executive and a large fraction of the policy-making bodies. These resources could (and should) be used to develop more meaningful and relevant educational programmes where emphasis on academic rewards is minimized, and also to train technicians and innovators.

As long as present Indian educational policies are retained, Dr Parthasarathi's hope that developed countries should produce professionals whose training is relevant to the Indian scene sounds naive. But the present situation could be exploited to retain or bring back the best professionals, not the most "academically qualified", but those with innovative capabilities. This may require much more flexible methods of recruitment. The shortage of qualified professionals does not extend to all fields and to a large extent it is due to highly specialized and often superfluous training. With a proper educational background, many professionals could be utilized in related jobs after a short period of reorientation. Alternatively, on-the-job training facilities could be exploited. As for giving professionals "greater control over their own activities", this would make little contribution to the overall direction of any project or programme. A developing country can ill afford a set of uncoordinated research projects which are not goal-directed in coordination with the overall direction of the programme.

A mixed group of "technocrats" with wider perspectives and relevant educational background can be generated to coordinate various phases of development—planning, training, execution and utilization. At the moment, this is done by politicians and bureaucrats trained in liberal arts and office chores. As the responsibility of the individual appears to be as great as that of the government, incentives and other positive "promotional methods" could supplement the means proposed to curb the negative forces arising out of the "poverty and rigidity" of the intellectual environment. I am certain that in most cases the lack of incentive is not due to insufficient economic benefits.

I agree with Dr Parthasarathi that professionals away from home should be told about career opportunities in India, but even a thorough study of bulletins of leading research organizations is not going to help in this matter. An average graduate (even of a foreign university) is not equipped to interpret the jargon of statistics and publicity phrases. If a more realistic picture were presented in the bulletins, their propaganda purpose would not be served, but I see no need to create images; a realistic picture can be challenging enough.

From personal experience, the most frustrating aspect of this business of "contact" is the lack of response to enquiries. To twenty-two letters enquiring about the possibility of a job, I received only four answers (in ten months) informing me that there was none. There is no significant lack of qualified applicants in most fields, and lack of personnel in some highly specialized technical fields can be differentiated from the myth of a general lack of college trained personnel. Thus a flat appeal to foreign governments to bar educated persons from immigration is irresponsible. A clear distinction between degree holders and qualified professionals with actual (not potential) job openings should be drawn, especially for framing policies which would decrease the mobility of an individual.

The measures Dr Parthasarathi suggests seem to have little relevance in terms of developing indigenous solutions. Foreign trained *sahibs* (even those with high academic laurels) may be the jewels of the establishment, but little can be expected from them by an average Indian. At best, they can be Peace Corps volunteers whose efficiency is open to question. Modern technology is an alien force in most human societies (even though it is here to stay) and its compatibility with human nature has not been proved even for technologically developed societies.

However, its integration with other cross-cultural forces can be aided by relevant education and planning which encompass cultural, economic, and political aspects of a society.

Yours faithfully,

M. K. JAIN

Department of Chemistry,
Indiana University,
Bloomington, Indiana 47401

Pollution is a Dirty Word

SIR,—In the past couple of years I have read or heard about pollution of the atmosphere (by radioisotopes from fallout, by tobacco smoke, car exhausts, open-hearth fires, factory chimneys and coal-burning trains); of fresh waters (by toxic or inhibitory agents, such as detergents and DDT, and by nutrients or growth stimulants, such as nitrates and phosphates); of the sea (by chemical effluents, which may be toxic to plankton, by sewage, which promotes its growth, and by effluents from nuclear power plants, which tend to warm it); of the scenery (by beer tins and roadside hoardings); of the ether (by advertisements or rock music, according to taste); of the night sky (by the shine of street-lights); by the wrong kinds of microbes and viruses (causing disease or bad beer); by the wrong kinds of fish (a consequence of introduction or inferior husbandry); by the wrong kinds of genes (a consequence of miscegenation or inadequate eugenic measures); and of the world's population in general (by our own unlimited propagation). There are endless protests against chemical pollution, thermal pollution, sound pollution and light pollution. Any day now, I expect to read about calorie pollution (too much sugar in one's diet), verbal pollution (one of the specialties of contemporary USA), personnel pollution (too many half-occupied typists), negative thermal pollution (in draughty corridors) . . . *ad nauseam*.

As children, we used certain words to impress our parents. As adults, applicants for public funds to support our research or other favoured activities, we tend to continue this practice. But as scientists and craftsmen, we should not overuse or misuse our tools or our words until they become so bent or so blunt that they lose their efficacy. Let us not muck up our language, lest we also muddle our minds.

Yours faithfully,

RALPH A. LEWIN

Scripps Institution of Oceanography,
University of California,
La Jolla, California

Semi-in-vivo?

SIR,—Dr Green advocates (*Nature*, 229, 142; 1970) dispensing with the terms *in vivo* and *in vitro* which he notes are often misused as adjectives and only retain usage as adverbial phrases in the pen of the *cognoscenti*. Surely this is not the case—trained scientific writers use these and other terms correctly and ought not to be dubbed as *cognoscenti* for so doing.

He states that some editors "are prepared to turn a blind eye to the terms" suspiciously foreign sound and are prepared to admit them as current English usage. Others, more severe, by clapping the terms in italics, clearly still regard them as aliens against whom the innocent reader must be warned". Surely the italics are used for the reason that in many publications all Latin terms enjoy italics.

He says that "'semi-in-vivo' experiments have recently been threatened". It is an interesting question—perhaps Dr Green or some other reader may be able to suggest a suitable term for use when reporting results of effects obtained when human or animal tissue cultures are used in contradistinction to those obtained from direct clinical application.

Yours faithfully,

DEIRDRE C. O'DONOGHUE-MAGUIRE

Connaught Medical Research
Laboratories,
1755 Steeles Avenue West,
Willowdale, Ontario

Expensive Meat

SIR,—The figures for the costs of tissue cell culture media given by Dr Moore (*Nature*, 230, 133; 1971) need amendment. Our work¹⁻³ has resulted in the development of defined media which makes the culture of suspended mammalian tissue cells much more predictable, and capable of much higher cell population densities and yields than was previously the case. Also, the abolition of serum from the medium has removed the most expensive ingredient which was a major obstacle to large scale culture.

A comparison of the costs of media is given in the Table.

Our costs are based on that of fine laboratory chemicals, hence for large scale usage the cost could be very much less. Also we know that yields could be considerably increased. Whether or not these costs would be comparable with that of best beef is at least debatable. On the grounds of rates of production there

would be an enormous gain by the use of cell culture. Under optimum conditions in continuous-flow culture the cell mass will reproduce itself in 4 days in defined medium, whereas I believe it takes about two years to produce a crop of bullocks. Dr Moore also seems to imply that there would be an ethical problem about meat production by tissue culture. But surely tissue cell culture could be the only solution to the ethical problems raised by modern battery production of farm animals.

Yours faithfully,

S. J. PIRT

Microbiology Department,
Queen Elizabeth College,
University of London

¹ Birch, J. R., and Pirt, S. J., *J. Cell Sci.*, 7, 661 (1970).

² Blaker, G. J., and Pirt, S. J., *J. Cell Sci.*, 8 (1971).

³ Blaker, G. J., Birch, J. R., and Pirt, S. J., *J. Cell Sci.* (in the press).

	This laboratory Mouse LS cells	Human HeLa cells	Dr Moore Human lymphocytes	Mouse cells
Maximum cell concentration (g wet weight/l.)*	4.4†	4.4†	0.8	2
Cost/kg wet cell weight * (£ sterling)	35.3	72.2	1,040	—

* I take it that Dr Moore's mass data refer to wet cell weights.

† 0.8 g dry weight.

Obituaries

Dr Geoffrey Bourne

GEOFFREY BOURNE, physician and cardiologist of St Bartholomew's Hospital, London, and author of a number of medical books, died in December 1970 at the age of seventy-seven.

After entering St Bartholomew's Hospital in 1912 with an entrance scholarship in arts, his very great ability was immediately shown when he won almost all the scholarships and prizes that were available. His distinguished career at the hospital continued until his retirement in 1959, and can best be summed up by the final words of his own book, *We Met at Barts* (1963), as "forty-seven years of association with an institution that had given me countless blessings, no regrets, wonderful friends and a professional experience beyond price".

As a physician and cardiologist, Geoffrey Bourne wrote many original papers contributing to the advancement of medical knowledge. He also sensed that heart patients would benefit from an explanation in lay terms of the problems of heart disease—his book *Heart Disease*

has been a great help to many people. In addition, his qualities as a physician included a great ability to observe and remember the patients of the hospital.

He was an active member of the British Cardiac Society, an interest that continued until the time of his death. His long association with the United States, which started with a Rockefeller Fellowship and which was strengthened by his happy marriage to Margherita Contonio of New Orleans, led him to found the Horseshoe Club to promote Anglo-American fellowship and the interchange of medical men between the two countries.

As a teacher of medicine, Geoffrey Bourne won great affection. His skill and interest are exemplified in his book *The Principles of Clinical Pathology in Practice*. His insight into the teaching methods of others made him concentrate on establishing his own diagnoses on fundamental principles and his affection for his students made him give much time to the Barts Journal.

Outside the realms of medicine he was equally outstanding. His interests included music and painting and his

performance in both these spheres gave much pleasure to others. He was a keen fly fisherman and his interest in natural history and country lore was centred largely round the cottage in Sussex where he spent many happy months over a period of fifty years. At the time of his death, he was finishing a book about this part of his life. An earlier book, *Return to Reason* (1942), showed his desire to bring sanity into politics.

After the death of his first wife in 1952, he married Patricia McCready who brought further happiness to his home life and who helped him to write the books for which they will both be so well remembered. In recording his skill, understanding and love for future generations, these books will temper the sense of loss shared by the thousands who were lucky enough to have been in personal contact with him.

Mr C. R. Barber

MR C. R. BARBER, a leading authority on temperature measurement and one of the principal architects of the Inter-

national Practical Temperature Scale of 1968, died on March 12, at the age of sixty-one.

Mr Barber's entire career was spent at the National Physical Laboratory. Joining it 44 years ago in a junior grade, he studied part-time for a degree and graduated in 1932. His early work was on the establishment and reproducibility of the 1927 International Temperature Scale at high temperatures. During the war he was involved in a range of difficult practical problems of temperature measurement. For example, he established that the rotating blades in the early Whittle jet engine were operating at unexpectedly high temperatures, a factor contributing to blade fracture. His interest in a "quick-immersion" thermocouple technique for measuring the temperature of molten steel led to his publishing extensive reference tables, still in use today, for platinum/platinum-rhodium thermocouples.

In the late 1940s Barber was appointed a member of the Consultative Committee for Thermometry (one of the advisory committees of the International Committee of Weights and Measures). In the following years he demonstrated, for example, the greater reproducibility of temperature at the triple point of water compared with the ice point; this led to the triple point being adopted internationally as the single defining point of the temperature scale. He designed a new platinum resistance thermometer of low time-constant and excellent stability, and played a major part in developing an improved form of Smith bridge, a bridge for the measurement of the resistance of platinum resistance thermometers. Copies of the new bridge were subsequently installed in numerous standardizing laboratories throughout the world.

Although Barber was to become increasingly involved in international comparisons and in committee work, both at national and international level, he remained active in experimental studies. For example, in 1955 he started a programme leading to the establishment of a low temperature scale based on helium gas thermometry. This work and related studies elsewhere provided the basis for the extension of the International Scale downward from 90.18 K to 13.81 K in the IPTS-1968. In the last few years Barber headed a research section at the NPL active on a wide range of studies in thermometry and related fields. He was awarded, in 1969, the Callendar Medal of the Institute of Measurement and Control in recognition of his life-time's work in thermometry.

Unassuming by nature, always friendly and helpful, Barber was a man

who was universally liked and highly regarded and whose death is a great loss to the subject of thermometry. His wife survives him.

Announcements

University News

Professor K. Keohane, Chelsea College of Science and Technology, has been appointed Royal Society Leverhulme visiting professor to the **Universidade Federale da Bahia**, Brazil.

Professor R. I. Mateles, Massachusetts Institute of Technology, has been appointed professor of applied microbiology at the **Hebrew University—Hadassah Medical School**, Jerusalem, and director of the Fermentation Unit, which is operated jointly by the University and by the Israeli Government.

Professor J. N. Walton, professor of neurology in the Department of Medicine, has been appointed dean of medicine in the **University of Newcastle upon Tyne**.

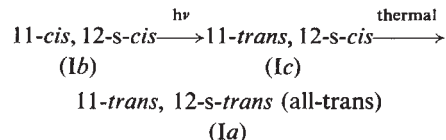
Miscellaneous

Dr Ira E. Puddington, director of the Division of Chemistry, National Research Council, Ottawa, has been awarded the Montreal medal of the **Chemical Institute of Canada**. The Institute's Merck Sharp and Dohme lecture award has been won by **Dr J. B. Stothers**, University of Western Ontario.

The Symons memorial gold medal of the **Royal Meteorological Society** has been awarded to **Mr J. S. Sawyer**, director of research, Meteorological Office, for his contributions to synoptic and dynamical meteorology. The Fitzroy prize has been awarded to **Dr R. C. Rainey**, the L. F. Richardson prize jointly to **Dr A. J. Gadd** and **J. F. Keers**, and the Darton prize to **Dr W. T. Roach**.

CORRIGENDUM. In the article "Late Australopithecine from Baringo District, Kenya" by J. Carney, A. Hill, J. A. Miller and A. Walker (*Nature*, **230**, 509; 1971), the comments on tooth wear (p. 514) should read as follows: "M² has only the tips of the metacone and hypocone worn, and M³, although fully formed, is unerupted".

ERRATUM. In the article "Implications of Torsional Potential of Retinal Isomers for Visual Excitation" by B. Honig and M. Karplus (*Nature*, **229**, 558; 1971), the following corrections should be made. The second sentence in the fourth paragraph of the first column of p. 560 should read: "...; the calculated values are -2,016 cm⁻¹ relative to the distorted 11-*cis*, 12-*s-cis* retinal and ...". The fourth sentence of the same paragraph should read: "The series of reactions



would correspond to the observed changes in the visual pigment spectrum ...". In the caption of Fig. 1, the second sentence should read: "... with respect to rotation about C₁₀-C₁₁ and C₁₁-C₁₂; ...". In text figure (a), the H at positions 9 and 13 should read CH₃, and in text figure (b) the CH at position 13 should read CH₃.

British Diary

Monday, May 10

High-Frequency Cables (5.30 p.m.) Professor H. E. M. Barlow, University of London, in the Botany Theatre, University College London, Gower Street, London WC1.

Some New Observations on the Pathology and Immunology of Mucous Membrane Infections with Particular Reference to *Bordetella pertussis* (5 p.m.) Dr L. B. Holt, University of London, at St Mary's Hospital Medical School, Wright-Fleming Institute, Norfolk Place, London W2.

The Future Development of Power Semiconductor Switching (2.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

The Scope of Zoology (5 p.m.) Professor D. Bellamy, University College Cardiff, in the Botany Lecture Theatre, Main College Building, University College, Cathays Park, Cardiff. (Inaugural Lecture.)

Tuesday, May 11

Quantum Electrodynamics and other Fields (5.30 p.m.) Professor T. W. B. Kibble, University of London, in Lecture Theatre A (Mechanical Engineering), Imperial College of Science and Technology, London SW7.

Social Policy in Drug Dependence (8 p.m.) Professor Morton Miller, Institute for the Study of Drug Dependence, jointly with the Society for the Study of Addiction, at the Botany Theatre, University College London, Gower Street, London WC1.

Some Contributions of Radioautography to the Cytochemistry of the Aminergic Neurons (5.30 p.m.) Professor J. Taxi, University of London, in the Anatomy Theatre, University College London, Gower Street, London WC1.

Some Observations on Badgers under Controlled Conditions, Mr P. Drabble; **The Ecology of the Adder**, Mr Ian Prestt; **The Seals of Macquarie Island** (film) Zoological Society of London, at the Zoological Gardens, Regent's Park, London NW1.

The Role of Rock Mechanics in the Reconnaissance of Rock Foundation (5.30 p.m.) M. Pierre Londe, University of London, in the Mining Lecture Theatre, Imperial College of Science and Technology, London SW7.

Visual Prosthesis—an Implanted Electrical Aid for the Sightless (5.30 p.m.) Mr P. E. K. Donaldson, Institution of Electrical Engineers, jointly with the Institute of Measurement and Control, at Savoy Place, London WC2.

Wednesday, May 12

Advances in TV Colour Cameras (5.30 p.m.) Mr A. V. Lord, Institution of Electrical Engineers, at Savoy Place, London WC2.

Antigen Receptors on Lymphocytes (2 p.m.) Dr J. H. Humphrey, University of London, at the Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12.

INSPEC—The IEE's International Solution to the Information Problem (5.30 p.m.) Mr D. H. Barlow, Institution of Electrical Engineers, at Savoy Place, London WC2.

Motorway and High Speed Road Surveillance and Control (6 p.m.) Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

Nutritive Consequences of Affluence (6.15 p.m.) Society of Chemical Industry, Food Group, at 14 Belgrave Square, London SW1.

Water Seepage in Rock Slopes (5.30 p.m.) M. Pierre Londe, University of London, in the Mining Lecture Theatre, Imperial College of Science and Technology, London SW7.

Thursday, May 13

Analysis of the Stability of Rock Slopes (5.30 p.m.) M. Pierre Londe, University of London, in the Mining Lecture Theatre, Imperial College of Science and Technology, London SW7.

Notes to Authors

Will contributors to *Nature* follow as far as possible the general style of the journal, and in particular the following rules:

1. Manuscripts should be typed in double spacing and submitted in duplicate. Two copies of all figures should be included, one for the printers and one for the referee.

2. References are indicated by superscript numbers in the order in which they appear in the text, and have the following form:

¹ Sargent, W. L. W., and Searle, L., *Astrophys. J. Lett.*, **162**, L155 (1970).

² Fraenkel-Conrat, H., in *Comprehensive Biochemistry* (edit. by Florkin, M., and Stotz, E. H.), **7**, 75 (Elsevier, Amsterdam, 1963).

"Unpublished work", "Private communication" and "Work in preparation" should not be cited as references but may be incorporated in the text. Each reference number should refer to an individual work which has either been published or is in the press.

3. Although the text of longer articles is usually interrupted by headings at suitable intervals, these should be short and informative and not merely "Introduction", "Results", or "Conclusion".

4. Units should either conform to the SI system or be spelled out in full.

A fuller guide can be had from the *Nature* offices in London and Washington.

Galileo; Isaac Newton; John Dalton (1 p.m. films) Royal Institution, at 21 Albemarle Street, London W1.

Landscape Maintenance and Conservation (8.15 p.m.) Professor W. Haber, University of London, at Wye College, near Ashford, Kent.

Ore Mineralogy in the Mining Industry (5.30 p.m.) Professor A. Millman, University College Cardiff, in the Large Lecture Theatre, Mineral Exploitation Department, Newport Road, Cardiff. (Inaugural Lecture.)

Friday, May 14

Biochemical Properties of Transplantation Antigens (5.30 p.m.) Dr S. Nathenson, University of London Hospital Medical College, Turner Street, London E1.

Earthing of Industrial Power Systems (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Singlet Molecular Oxygen (1 p.m.) Dr R. P. Wayne, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

The Survival of Species (9 p.m.) Mr Peter Scott, CBE, Royal Institution, at 21 Albemarle Street, London W1.

Monday, May 17

Celebrations to Mark the Centenary of the Foundation of the Institution of Electrical Engineers (three days, London).

Constructivity and Quantization (5.30 p.m.) Professor C. Kilmister, British Society for the Philosophy of Science, in the Joint Staff Common Room, University College London, Gower Street, London WC1.

Tumour Viruses and Cell Growth (5 p.m.) Dr M. G. P. Stoker, University of London, at St Mary's Hospital Medical School, Wright-Fleming Institute, Norfolk Place, London W2.

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.

The direct postal price per subscription is:

12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	U.S.A.	Airfreight Canada
Nature (Friday)	£14	\$48	\$52
Nature+			
Nature Physical Science	£24	\$83	\$90
Nature+			
Nature New Biology	£24	\$83	\$90
All three editions	£29 10s	\$108	\$116
Annual Index	£1	\$3	\$3

* Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Editorial and Publishing Offices of "NATURE"

MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2R 3LF

Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number: Basingstoke 5431

Advertisements only should be addressed to
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN LONDON WC2A 2ED
Telephone 01-242 6264/01-405 4743
Telegrams: Textualist London WC2A 2ED

Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, May 7, 1971